EXPERIMENTAL TAXONOMY IN THE FAMILY EPACRIDACEAE

by

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DECLARATION

Except as stated herein, this thesis contains no material which has been accepted for the award of any other degree or diploma in any university and to the best of my knowledge and belief, the thesis contains no copy or paraphrase of material previously published or written by another person, except where due reference is made in the text of the thesis.

Sandra Jean Jarman

PREFACE

The thesis is arranged in three parts -

PART I introduces the family and presents the objectives of the thesis.

PART II involves the chemical investigation of the Epacridaceae and discusses taxonomic and phylogenetic aspects in the light of flavonoid chemistry.
Numerical treatment of the data is also included.

PART III discusses the taxonomic problems in three specific genera.

Three appendices are attached -

Appendix A describes the experimental procedures and includes supplementary chemical data.

Appendix B describes numerical procedures and includes numerical data.

Appendix C contains species descriptions and a floral key.

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SUMMARY

One hundred and thirteen species or varieties were examined for the presence of flavonoids in a chemotaxonomic study of the Epacridaceae. Species were obtained from Tasmania, Victoria, New South Wales, New Zealand and South America. Accessions from outside Tasmania contained the same general complement of flavonoids as the Tasmanian species.

The characteristic flavonoids of the Epacridaceae are anthocyanins, flavonols and leucoanthocyanidins but dihydroflavones and chalcones were also present. Five common aglycones were identified i.e. cyanidin, delphinidin, kaempferol, quercetin and myricetin but pelargonidin, malvidin and possibly isorhamnetin were also present. The results suggest that myricetin may be more widespread in nature than is normally accepted, its apparent rarity being due to its lability under the conditions used in its detection. Sugars involved in glycosylation included galactose, glucose, arabinose, xylose, rhamnose and glucuronic acid. Substitution occurred characteristically at position 3 but two flavonols were glycosylated at position 5. Methylation was rare, and 3,5-diglycosylation in anthocyanins and 3,7-diglycosylation in flavonols was not observed although 3-diglycosides were common. Four pigments including cyanidin- and delphinidin-3-rhamnosylgalactoside, cyanidin-3-xylosylarabinoside and quercetin-3-xylosylrhamnoside are reported for the first time. Several species-specific compounds were observed and particularly with flavonols, chromatographic patterns proved valuable in species identification.

Glycosides were found to have greater importance taxonomically than aglycones and the ratio of the major glycosidic types appears to have phylogenetic as well as taxonomic significance. The results suggest that anthocyanin and flavonol galactosides are more advanced in the family than anthocyanin arabinosides and flavonol glucuronides respectively. A correlation between chemical and cytological data enabled an evolutionary index to be calculated for 36 species, and a chemical comparison between the two subfamilies supports the opinion that the Styphelieae is more advanced than the Epacrideae.

Three genera, *Epacris*, *Cyathodes* and *Monotoca*, have been examined in more detail than the remaining genera, and the contribution of flavonoid compounds to their taxonomy is discussed. Two new species are described for the first time.

Several numerical analyses were carried out on the chemical data and a discussion of the results is given.

A comparison between the Epacridaceae and published data for the related family, the Ericaceae, reveals some pronounced chemical differences, although the two are linked by the common occurrence of anthocyanin and flavonol galactosides and arabinosides. Evidence suggests that evolution towards more complex aglycones has occurred in the Ericaceae whereas greater variation in glycosylation is apparent in the Epacridaceae.

PART I

THE EPACRIDACEAE

A. CLASSIFICATION OF THE EPACRIDACEAE

In the natural classification of plants, the family Epacridaceae is placed in the order Ericales where it occupies a position adjacent to the Ericaceae. Although many plant classifications have been proposed since the first Epacrid species were encountered (c. 1776), the systematic position of the family has remained virtually unchanged, and its close affinity with the Ericaceae is widely accepted (Drude - 1889, Rendle - 1952, Cronquist - 1968, Takhtajan - 1969, Hutchinson - 1973, etc.).

To demonstrate the relative position of the family among the Angiosperms, a brief outline of the classification proposed recently by Cronquist is given below. With some minor differences, the system described by Cronquist (1968) is similar to that of Takhtajan (1969).

The class Angiospermae is divided into ten sub-classes, of which four are collectively termed the Liliatae and represent the Monocotyledones, while the remaining six, the Magnoliatae, represent the Dicotyledones. The general relationships between the six sub-classes of the Magnoliatae *viz.* the Magnolidae, the Hammamelidae, the Caryophyllidae, the Rosidae, the Dillenidae and the Asteridae, are illustrated in the following diagram.

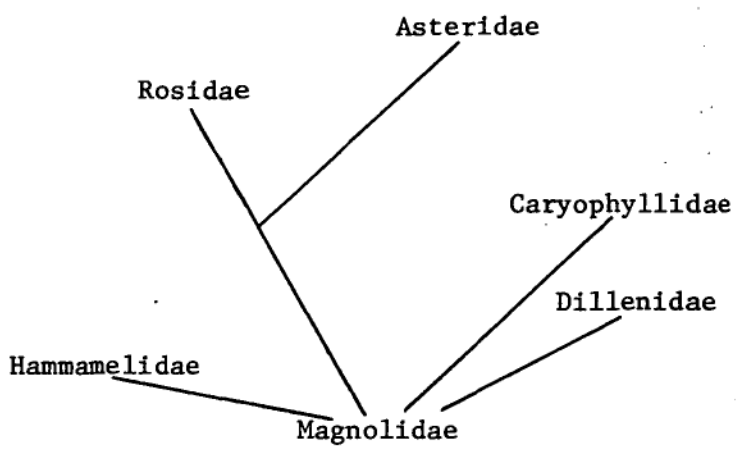


Fig. 1. The Magnoliatae (taken from Cronquist - 1968).

The Epacridaceae is placed in the order Ericales which is included in the sub-class Dillenidae along with eleven other orders. The probable relationships between the orders are shown in the following diagram.

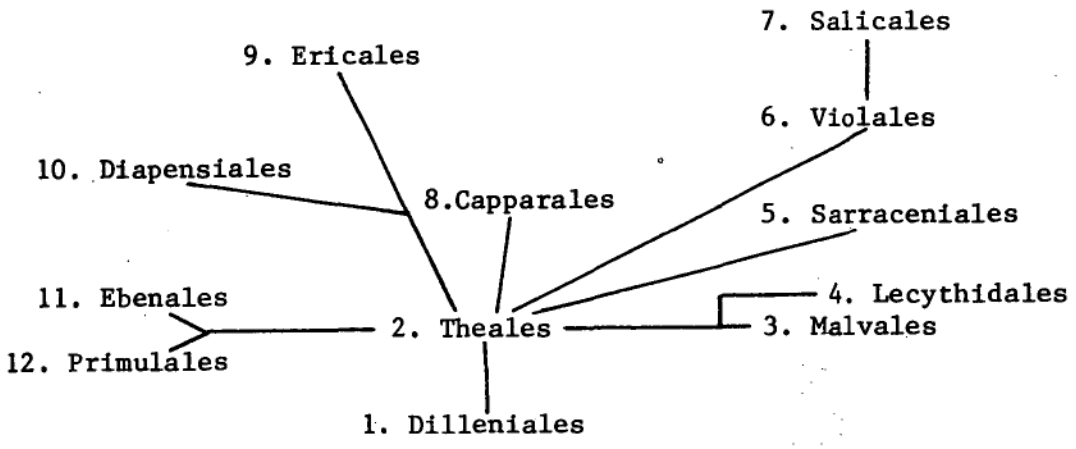


Fig. 2. The sub-class Dillenidae (taken from Cronquist - 1968).

The number of families comprising the order Ericales varies according to different taxonomists. Cronquist includes seven families - the Cyrillaceae (13 species), the Clethraceae (30 spp.), the Ericaceae (more than 2500 spp.), the Epacridaceae (c. 410 spp.), the Empetraceae (9 spp.), the Pyrolaceae (45 spp.) and the Monotropaceae (30 spp.).

Two of the more primitive families, the Cyrillaceae and the Clethraceae, are thought to be intermediate between the Ericales and the Theales. A third family, the Actinidiaceae, forms a link between these two orders and the Dilleniales, but while Takhtajan (1969) places it in the Ericales, Cronquist feels that it is better accomodated in the Theales. A small order, the Diapensiales, containing a single family, the Diapensiaceae, is also closely related to the Ericales and has been included in it by some taxonomists. According to Takhtajan, the Diapensiaceae is especially close to the Epacridaceae.

1. Relationship to the Ericaceae

Within the Ericales, the Ericaceae is considered nearest to the Epacridaceae. The two families occupy a similar ecological niche but on different continents. In Australia, the Epacridaceae is widespread whereas the number of Ericaceous species is low (4 spp.) and their

distribution is very restricted. J.D.Hooker (1860) remarks -

> "The replacement of the Ericeae of all temperate regions of the world by the Epacrideae in Australia, is one of the most singular phenomena in the geographical distribution of plants; and what is even more singular, the few Australian Ericeae which do exist are confined to the mountains of Tasmania, with the exception of one of them, which has recently been found by Mueller on the Australian Alps.".

The two families are separated taxonomically by relatively minor characters. In the Epacridaceae, the stamens are usually epipetalous and equal in number to the petals and sepals (excepting in *Oligarrhena* where the number of stamens is less). The filaments are usually fused to the corolla tube, the anthers are rarely appendaged and anther dehiscence is through a single longitudional slit. In the Ericaceae, the stamens are hypopetalous and there are usually twice as many as there are petals and sepals. The filaments are free, the anthers are usually appendaged and dehiscence is usually through an apical pore. Differences between the two families also occur in leaf venation. For example, species from the Epacridaceae usually have parallel or palmate venation whist those from the Ericaceae typically possess reticulate venation.

In spite of their minor differences, taxonomists have not attempted to unite the two families. In a recent numerical evaluation of character differences and similarities, Watson *et al.* (1967) found that the two separated very early in the dendrogram suggesting that whilst there were no conspicuous differences involved, the division into separate families was nevertheless a real one.

Three monotypic genera exhibit intermediate forms between the Epacridaceae and the Ericaceae. *Lebetanthus* and *Prionotes* link the two families in possessing five free, hypogynous stamens, longitudional anther dehiscence, unappendaged anthers and reticulate venation in the leaves. In 1915, Skottsberg (cited in Paterson - 1961 and Copeland - 1954) reduced *Lebetanthus* to *Prionotes* and placed the "new" *Prionotes* in the Ericaceae. However, most taxonomists prefer to maintain the two as separate genera of the Epacridaceae and their removal from the Ericaceae has recently been supported by Stevens (1971). In 1948, Burtt removed a third genus, *Wittsteinia*, from the tribe Vaccinioideae of

of the Ericaceae and placed it with *Prionotes* and *Lebetanthus* in Drude's tribe Prionotae of the Epacridaceae. The genus fits poorly into either family, and certainly the presence of an inferior ovary is quite unique among the Epacrids. However, until recently, *Wittsteinia* has generally been accepted in the Epacridaceae although its position in the Prionotae has been opposed by Watson (1967) who suggests that its differences are sufficient for the genus to merit tribal recognition. Stevens (1971) published a new classification for the Ericaceae in which he included *Wittsteinia* after demonstrating by appropriate comparisons that it was closer to this family than to the Epacridaceae.

An alternative treatment of *Prionotes*, *Lebetanthus* and *Wittsteinia* has been presented by Hutchinson (1973) who created a new family, the Prionotaceae, in which he places all three genera.

In this thesis, Stevens' treatment of *Wittsteinia* has been followed and *Prionotes* and *Lebetanthus* have been maintained as distinct genera of the sub-family Epacrideae (Epacridaceae).

Of the two families, the Epacridaceae is generally considered more specialized than the Ericaceae. Paterson (1961) has suggested that they may be related by a common ancestor or that the Epacridaceae may have been derived from the Ericaceae at some very early stage in their evolution. The former view has also been expressed by Venkata Rao (1961) and the latter by Copeland (1954).

The close morphological similarity between the subfamily Epacrideae (Epacridaceae) and the Ericoideae (Ericaceae) would seem to indicate a close relationship between these two groups in particular (Rendle - 1952, Smith-White - 1948, 1959b). The presence of tetrad pollen and the frequent occurrence of the haploid chromosome number, n = 13, was initially believed by Smith-White (1948) to support this view. However, his discovery (1955) of chromosome numbers n = 6 and 7 in *Sphenotoma* (Epacridaceae) has led him to revise his earlier opinion and deny any close relationship between the two groups at the higher chromosome level. Nevertheless, depending on the origin of chromosome numbers, it is possible that the two are related at the lower chromosome level.

Copeland (1954) has suggested a possible origin for the family from within the tribes Andromedeae and Gaultherieae of the Ericaceae (after Bentham - 1869), apparently because of their overlapping distribution with the Epacridaceae in Tasmania. Tasmanian representatives of these two groups produce tetrad pollen (McPhail - personal communication) and, like the Ericoideae and the Epacrideae, have a loculicidially

dehiscent capsule.

2. Taxonomic Divisions within the Epacridaceae

The Epacridaceae divides naturally into two large groups on the basis of reproductive characters and this primary division has received general recognition. However, subsequent divisions of the group are a constant source of controversy.

The first major work involving the family was published in 1810 by Robert Brown. He introduced the Order Epacrideae and placed it next to the Order Ericeae in the natural classification. Two sections, twenty four genera and one hundred and forty six species were described. His major division was based on fruit and ovary characters, with Section I containing all Epacrid species which produced an indehiscent, drupaceous fruit, single ovules in each loculus and with the style attenuate with the ovary. In Section II, he placed those species which produced a loculicidially dehiscent capsule, many ovules in each loculus and a style inserted in a central depression of the ovary. The characters used to distinguish the two groups are constant and easy to determine.

Brown's classification received wide acceptance and with slight modifications as new species were discovered, was followed by prominent botanists such as de Candolle, Eichler, Endlicher, Lindley, Bentham and Hooker, etc. Even botanists who did not support Brown's classification unreservedly used it as a reference point for their own work.

In 1867, von Mueller published a classification in which the delimitation of several genera, and in particular *Styphelia*, differed significantly from Brown's. When the genus *Styphelia* was first described by Smith very few Epacrids with drupaceous fruit were known and most were included in *Styphelia*. As the discovery of new species continued, the morphological diversity within the group became apparent. Robert Brown proposed that the genus should be divided into a number of smaller genera, one of which would retain the name *Styphelia*. At the same time, he suggested that others might prefer a single genus only, which included sub-generic divisions corresponding to his smaller groups. This latter idea received little support until it was adopted by von Mueller, who expanded *Styphelia* to include ten genera but retained the same divisions at a lower level. In support of his classification, von Mueller claimed that frequently the characters used to separate the smaller genera could not be relied upon, and several

species e.g. *Styphelia melaleucoides, Cyathodes petiolaris, Astroloma baxteri,* etc. possessed intermediate characters. In addition, he maintained that even as a single comprehensive genus, *Styphelia* would contain fewer species than some other genera in the plant kingdom e.g. *Acacia* (Leguminosae).

The reasons put forward by von Mueller are discussed in Bentham's "Flora Australiensis" (1869). While agreeing with the validity of von Mueller's statements, Bentham has argued that the large genera to which von Mueller was referring showed great uniformity in floral characters but this was not the case with *Styphelia*. With respect to inconsistent characters used for the delimitation of genera, Bentham states that "if in some characters hitherto given, some have failed, others have been brought forward in their support" and in the plant kingdom "there are few, if any, large genera which are not more or less connected to others by intermediate species". Finally, he suggests that since it is really only a matter of convenience whether Brown's or von Mueller's classification is accepted, then it would be preferable to follow the classification more widely used and in doing so, avoid a great many unnecessary name changes. In spite of the logic behind this argument, classifications based on both schemes are in use today.

As well as proposing the large comprehensive genus *Styphelia*, von Mueller united *Pentachondra, Decaspora* and *Trochocarpa* to form the single genus *Trochocarpa*. The inclusion of *Decaspora* with *Trochocarpa* is generally accepted but most taxonomists feel that *Pentachondra* is sufficiently different to be maintained as a separate genus. Von Mueller also included *Archeria* with *Epacris* but this move gained little support. He suggested no changes at the tribal level.

In 1869 and 1876, a classification was outlined by Bentham and by Bentham and Hooker respectively. It was based on Brown's scheme but included several minor modifications arising from the discovery of new species.

In 1889, Drude created a new tribe, the Prionotae, in which he placed the two anomalous genera, *Prionotes* and *Lebetanthus*, after removing them from the tribe Epacrideae. This move is accepted by few taxonomists and has gained no support from cytology (Venkata Rao - 1959) or anatomical work (Watson - 1967), at least in the case of *Prionotes*.

A comparison of the classifications proposed by Brown, von Mueller, Bentham and Hooker, and Drude is shown in Table 1.

In 1967, a completely new taxonomic structure for the family

(1810) Brown	(1876) Bentham & Hooker	(1867) von Mueller	(1889) Drude
Tribe Styphelieae			
Styphelia	*Styphelia*	*Styphelia*	*Styphelia* including *Cyathopsis*
Astroloma	*Astroloma*		
Stenanthera			
Melichrus	*Melichrus*		
Cyathodes	*Cyathodes*		
Leucopogon	*Leucopogon*		
Lissanthe	*Lissanthe*		
	Coleanthera		*Coleanthera*
	Conostephium		*Conostephium*
	Brachyloma		*Brachyloma*
Acrotriche	*Acrotriche*		*Acrotriche*
Monotoca	*Monotoca*	*Monotoca*	*Monotoca*
Needhamia	*Needhamia*	*Needhamia*	*Needhamia*
Oligarrhena	*Oligarrhena*	*Oligarrhena*	*Oligarrhena*
Decaspora	*Trochocarpa*	*Trochocarpa*	*Trochocarpa*
Trochocarpa			
Pentachondra	*Pentachondra*		*Pentachondra*
	Cyathopsis		
Tribe Epacrideae			
Epacris	*Epacris*	*Epacris*	*Epacris*
	Archeria		
Lysinema	*Lysinema*	*Lysinema*	*Lysinema*
			Woollsia
Cosmelia	*Cosmelia*	*Cosmelia*	*Cosmelia*
Andersonia	*Andersonia*		
Ponceletia	*Sprengelia*	*Sprengelia*	*Sprengelia*
Sprengelia		*Ponceletia*	
Cystanthe	*Richea*	*Richea*	*Richea*
Richea			
Dracophyllum	*Dracophyllum*	*Dracophyllum*	*Dracophyllum*
		Sphenotoma	*Sphenotoma*
			Tribe Prionotae
Prionotes	*Prionotes*	*Prionotes*	*Prionotes*
	Lebetanthus		*Lebetanthus*
Additional genera			
Rupicola			
Choristemon			
Decatoca			

Table 1. A comparison between four classifications of the Epacridaceae.

was proposed by Watson. As well as having additional information (anatomical data) not available to earlier taxonomists, Watson has obviously weighed the importance of various characters quite differently. He has questioned the significance of the conventional separation into two groups using fruit and ovary characters and instead has based his primary separation on vegetative characters. Two subfamilies and six tribes are included in his scheme. Drude's tribe Prionotae is not recognised but the monotypic genus *Wittsteinia* is included in the family and is placed in a new tribe, the Wittsteinieae. Two other new tribes, the Oligarrheneae and the Needhamielleae, also contain only a single species each but Watson defends the existence of these tribes on their very unusual and different characteristics. He maintains that the inclusion of these three species into any of the other tribes would disrupt the uniformity of the larger groups whereas by separating them an indication of their unusual characteristics is immediately apparent. Watson's classification is shown below in Table 2.

Subfamily Richeoideae

Richea, Dracophyllum, Sphenotoma

Subfamily Epacridoideae

Tribe Cosmelieae

Cosmelia, Sprengelia, Andersonia

Tribe Oligarrheneae

Oligarrhena

Tribe Wittsteinieae

Wittsteinia

Tribe Needhamielleae

Needhamia

Tribe Epacrideae

Epacris, Lysinema, Prionotes, Lebetanthus, Rupicola, Archeria

Tribe Styphelieae

Styphelia, Coleanthera, Conostephium, Astroloma, Melichrus, Cyathodes, Pentachondra, Brachyloma, Lissanthe, Leucopogon, Acrotriche, Monotoca, Decatoca, Trochocarpa, Cyathopsis.

Table 2. A new classification for the Epacridaceae suggested by Watson (1967).

Although the classification proposed by Watson appears substantially different from earlier classifications, the affinities and peculiarities represented in his groups were observed by the earliest Epacrid taxonomists. In his introduction to the Order, Brown (1810) noted the presence of three groups in Section II which could be recognised from their vegetative characters. These groups correspond to Watson's subfamily Richioideae and tribes Cosmelieae and Epacrideae. The same three divisions were observed by Bentham (1869). The unusual characteristics of *Needhamia* and *Oligarrhena* has led to their being maintained as monotypic genera and *Wittsteinia* is so different from other genera that it is frequently excluded from the family. Whether or not Watson's classification becomes generally accepted, the divisions represented in his scheme are not easily disputed.

At present, there is no completely comprehensive treatment of the family available. In 1869, a detailed account of Australian species was published by Bentham but representatives outside of Australia were not considered. Details of all genera, including general descriptions and distribution, were published by Bentham and Hooker in 1876 and by Drude in 1889, but individual species were not treated. Since these works, no large scale taxonomic revision of the family has been undertaken in spite of the many new species which have been discovered. Regional Floras have been compiled but many of these now require revision also.

Following Curtis (1963), the classification of Bentham and Hooker (1876) has been adopted in this thesis. This classification has been accepted by many taxonomists over the years including Moore and Betche (1893), Rodway (1903), Black (1922 - 29), Ewart (1930), Blackall and Grieve (1965), Burbidge and Gray (1970), Willis (1972), etc. It has received support from studies in chromosome numbers and pollen development (Smith-White - 1955), pollen morphology (Franks and Watson - 1963) and floral morphology (Paterson - 1961). Several modifications of the classification have been necessary to include species and genera not recorded as early as 1876. In addition, in this thesis, two other alterations have been made i.e. the genus *Sphenotoma* has been retained separately from *Dracophyllum* in accordance with the recently published Western Australian Flora (Blackall and Grieve - 1965) and *Woollsia* has been removed from *Lysinema*. The separation of these genera is supported by morphological differences and by their disjunct

distribution (*Sphenotoma* and *Lysinema* in Western Australia and *Dracophyllum* and *Woollsia* in eastern Australia). In further support, Watson (1962) has demonstrated clear stomatal differences between *Sphenotoma* and *Dracophyllum* and Smith-White (1955) has recorded differences in chromosome number between *Lysinema* (n = 12) and *Woollsia* (n = 13).

3. Generic Affinities

A very brief treatment of affinities between genera is given by Bentham (1869) whose observations are mostly based on morphological characters. Although his work is more than 100 years old, few significant changes to the proposed affinities have been made since its publication. *Prionotes* and *Lebetanthus* are considered close and both have strong affinities with *Epacris*. They differ from *Epacris* in the presence of reticulate leaf venation and hypogynous stamens. Both *Lysinema* and *Archeria* are also related to *Epacris*, the former differing in aestivation whilst the latter has fewer bracts on the pedicel. Although *Cosmelia* was also considered close to *Epacris* by Bentham, Watson (1967) has placed it with the two allied genera, *Sprengelia* and *Andersonia*, on the basis of its vegetative characters. *Richea*, *Dracophyllum* and *Sphenotoma* form a separate group in which *Dracophyllum* and *Sphenotoma* are particularly close and were included in the one genus by Bentham.

In the Styphelieae, *Cyathodes*, *Lissanthe* and *Leucopogon* are particularly close but differ in minor inflorescence characters. *Lissanthe* is separated from *Leucopogon* soley by the absence of hairs on the corolla lobes. *Brachyloma* is close to *Lissanthe* and *Leucopogon* but is readily distinguished by the imbricate aestivation of the corolla lobes. *Astroloma* and *Monotoca* are also allied with *Leucopogon* and all three contain species with overlapping characteristics. *Styphelia* is related to *Astroloma* but differs particularly in the revolute corolla lobes and the exerted anthers. From a study of floral anatomy, Paterson (1961) states that *Astroloma* and *Melichrus* seem to be closer to *Styphelia* than do *Leucopogon* and the genera allied to it.

Pentachondra and *Trochocarpa* are unusual in producing a stone which separates into pyrenes. The two genera differ in inflorescence characters and also in the number of pyrenes formed. There is some tendency for the stone to partially separate in *Acrotriche*, but not to the extent observed in *Pentachondra* and *Trochocarpa*.

Affinities among the genera are also apparent from anatomical studies carried out by Dormer (1945) and Watson (1962, 1967). Dormer described three groups in the family based on leaf morphology, nodal anatomy and pith structure. One of these groups was further divided by Watson, who also examined leaf anatomy and stomatal characteristics.

The broad pattern of affinities within the family, as represented by anatomical characters, may be seen in Table 3 in which a correlation between leaf fibre pattern, nodal anatomy and pith structure is given.

(a) The *Styphelia* group

Veins *Styphelia* pattern (except *Trochocarpa* and some *Leucopogon* spp.); nodes unilacunar; leaf bases narrow; pith homogeneous.

Styphelia, Coleanthera, Astroloma, Conostephium, Melichrus, Cyathodes, Pentachondra, Cyathopsis, Decatoca, Brachyloma, Lissanthe, Leucopogon, Acrotriche, Monotoca, Trochocarpa.

(b) The *Epacris* group

Veins *Epacris* pattern; nodes unilacunar; leaf bases narrow; pith homogeneous.

Epacris, Lysinema, Archeria, Rupicola, Prionotes, Lebetanthus, Woollsia

(c) The *Cosmelia* group

Veins *Cosmelia* pattern; nodes unilacunar; leaf bases sheathing; pith homogeneous.

Cosmelia, Sprengelia, Andersonia

(d) The *Richea* group

Veins *Richea* pattern; nodes tri- or multi-lacunar; leaf bases sheathing; pith heterogeneous.

Richea, Dracophyllum, Sphenotoma

(e) Special cases

(i) Veins *Epacris* pattern; leaf bases narrow; pith homogeneous; nodes unilacunar.

Needhamia, Oligarrhena

(ii) Veins with associated fibres; leaf bases narrow; pith homogeneous; nodes unilacunar.

Wittsteinia

Table 3. Correlation of leaf fibre pattern with nodal anatomy and pith structure taken from Watson (1967).

4. Phylogenetic Relationships

In the absence of a well defined fossil record, any attempts to trace the evolution of particular genera or species in the Epacridaceae

will always be accompanied by a strong element of speculation.

The Epacrideae is generally considered the more primitive of the two subfamilies because of its closer affinity to the Ericaceae and the occurrence of several characters considered relatively more primitive than in the Styphelieae i.e. the presence of a capsule, numerous ovules per loculus, free anthers in several species, the absence of any disturbance to the usual hermaphroditic breeding system, etc. Cytological (Smith-White - 1948) and morphological work (Paterson - 1961) has suggested a similar evolutionary status between the two groups.

Evolutionary trends among genera of the family are more difficult to establish but an examination of character progressions may prove valuable for this purpose. Although such progressions are applicable to the evolution of a particular character rather than to an actual genus, they must also reflect to a certain extent, the evolutionary changes occurring between the genera involved - those genera accumulating a majority of early stages in the development of several different characters are likely to be more primitive than those accumulating a majority of later stages. The practical application of this principle is limited however, since it is rarely possible to demonstrate unequivocally the direction or rate of evolution in a sequence of character states.

Apart from cytological work, it is difficult to obtain published information regarding established character progressions in the family. For this reason, the discussion given here is necessarily limited in scope but because of its relevance to chemical work (see p. 88), the subject is treated in some detail.

From cytological work carried out by Smith-White (1948, 1955, 1959a) and Venkata Rao (1961), it is possible to demonstrate a progressive series in both chromosome numbers and pollen development in the Epacridaceae. Variation in the breeding system of some species is also apparent.

(a) Chromosome numbers

In the following discussion, the number of chromosomes comprising the base number of a chromosome series will be designated by x whilst the haploid chromosome number will be given by n.

Haploid chromosome numbers occurring in the Epacridaceae include $n = 4, 6, 7, 8, 9, 10, 11, 13, 14, 16$ and 24 (Smith-White - 1955). These numbers do not represent a continuously increasing or decreasing sequence, but have been formed from polyploid and aneuploid changes on

the basic chromosome number. Smith-White (1955, 1959a, 1959b) believes that x = 4 and 6 are the basic chromosome numbers in the Styphelieae whilst x = 6 is the basic chromosome number in the Epacrideae. It follows from this that x = 6 is the most probable basic chromosome number for the whole family.

Chromosome numbers in the Styphelieae are more variable than in the Epacrideae. Polyploid series on the base numbers 4 and 6 are apparent i.e. n = 4, 8, 12, 16 and n = 6, 12, 24. Other haploid numbers occurring in the group i.e. 7, 9, 10, 11 and 13 are thought to have originated through aneuploid changes. It seems probable that n = 14 may have been derived from n = 7 by polyploidy. Haploid and base chromosome numbers given for the Styphelieae are shown in Table 4. With the exception of n = 13 for *Pentachondra* (Venkata Rao - 1961), the chromosome numbers have been taken from Smith-White (1955, 1959b).

Genus	n	x
Acrotriche	9	9
Astroloma	4, 7, 8, 12, 16	4, 7
Conostephium	8	4, 8
Cyathodes	9, 10, 12	
Leucopogon	4, 6, 7, 8, 10, 11 12, 14, 22, 24	4, 6, 11
Lissanthe	7, 14	7
Melichrus	8	4, 8
Monotoca	12	12
Pentachondra	13, 14	7
Styphelia	4	4
Trochocarpa	10	

Table 4. Haploid and base chromosome numbers in the Styphelieae.

In the Epacrideae, most genera are characterized by n = 13, but in *Sprengelia* and *Lysinema*, n = 12 is typical and for *Sphenotoma*, n = 6 and 7 have been found.

Smith-White (1955) considers that the direction of evolution follows a progressive increase in ploidy which is frequently accompanied by aneuploid changes. The presence of symmetrical karyotypes with n = 4 and highly asymmetrical karyotypes of n = 7 and 9 is used as evidence to support his hypothesis, in that Stebbins (1950 - see also 1971) has argued that asymmetrical karyotypes are likely to be derived. The association of n = 4 and 6, and polyploids on these two base numbers, with S-type pollen (see following section), also suggests that low

chromosome numbers represent the primitive condition.

(b) Pollen types

The single pollen grains produced by most Angiosperm families are rare in the Order Ericales. With the exception of the Clethraceae, families in the Order characteristically produce tetrad pollen. While this is generally true for the Epacridaceae, there is some diversity within the group. Three *Richea* species from the subfamily Epacrideae are reported to produce single pollen grains (Venkata Rao - 1961), otherwise tetrads are produced uniformly in the subfamily. Five different pollen types have been described in the subfamily Styphelieae. These are designated S-type (*Styphelia* type), S'-type (modified *Styphelia* type), V-type (variable tetrad type), P-type (*Pentachondra* type) and T-type (tetrad type).

S-type pollen is the most common in the Styphelieae. After meiosis in the pollen mother cell, all four nuclei are arranged regularly. Before cell walls are laid down, three nuclei migrate to one end of the cell and the fourth nucleus moves to or remains isolated at the opposite end. Cell walls are formed and although some development occurs in all four cells for a short time, very soon the three smaller cells cease to grow. They become compressed and abort, and at maturity, the tetrad resembles a single grain.

The pattern of development is similar in S' pollen but the four microspores are initially all equal in size i.e. there is no nuclear migration in the pollen mother cell. Slight development occurs in all four cells, but after a short time, three cells abort and subsequent growth of the tetrad is entirely due to the remaining cell. At maturity, S and S' pollen cannot be easily distinguished.

Smith-White (1959a) has recorded intermediate pollen types in which there is a variable degree of nuclear migration in the pollen mother cell. Hence, in some species, the distinction between S and S' is arbitrary. In order to avoid a subjective assessment of these pollen types, Smith-White introduces the term "monad" (M) to describe S and S' pollen collectively.

In V-type pollen, all four microspores are initially equal but none, one, two, three or all four may fail to develop. It forms a link between M and T-type pollen in that all degrees of variation have been found, from a modal frequency of monads to one of tetrads. V-type pollen has not been recorded in species with haploid chromosome numbers n = 4 or 6, or with multiples of these. It is associated with

aneuploidy and in some species, irregularities at meiosis have been detected.

In T-type pollen, all four microspores develop regularly. As with V-type pollen, it is not associated with n = 4 or 6, or with their direct multiples.

P-type pollen was not observed by Smith-White and has only been found in one endemic Tasmanian species i.e. *Pentachondra involucrata* (Venkata Rao - 1961). In P-type pollen, two of the four microspores regularly develop.

With no evidence to the contrary, it is logical to assume that these differing pollen types are related by modifications of the same basic developmental system. Smith-White has attempted to describe such a system that fits the experimental data, relates the development of each pollen type and does not conflict with known cytological and genetical processes (Smith-White - 1959a).

Smith-White (1959a) and Venkata Rao (1961) have suggested an evolutionary progression for pollen development in the Epacridaceae. However, the direction of evolution in this sequence is viewed differently by the two workers. Smith-White believes that S-type pollen, although originally derived from T-type pollen, represents the primitive condition in the Styphelieae, and originated at the same time as the subfamily or shortly afterwards. Development has been progressively modified, or broken down, to give S', V and T-type pollen. Where it occurs in the Styphelieae, T-type pollen represents the most recent condition.

Contrary to this, Venkata Rao traces the evolutionary progression in the opposite direction. Overall in the family, he believes that the single grains found in the subfamily Epacrideae represent the earliest pollen type. Failure of the four microspores to separate after meiosis has led to the development of tetrad pollen. In the subfamily Styphelieae, T-type pollen is the most primitive form and has been progressively modified to give V, P and finally, S-type pollen. Venkata Rao considers the elongated S-type pollen found in *Monotoca* to be a further modification of the more usual spherical shaped S-type. In his view, this pollen type is the most advanced in the family.

The presence of several pollen types in different genera has led Venkata Rao to suggest that monad pollen is polyphyletic, having arisen independently several times. However, this possibility is dismissed by Smith-White (1959a) who argues that the development of monad pollen is complex and would probably breakdown on several occasions rather than

develop a number of times. Smith-White maintains that if monad pollen were advanced, then the chromosome numbers, n = 7, 9 and 10 must be more primitive than n = 4 and 6, and therefore the latter must also have arisen independently on several occasions and always in association with monad pollen. He finds this theory completely unacceptable and instead, considers S-type pollen monophyletic and together with chromosome numbers, n = 4 and 6, representative of the primitive condition in the Styphelieae.

(c) Breeding system

Dioecism is considered by Hutchinson (1973) to be an advanced character. Outcrossing is obligatory and a high degree of recombination with accompanying genetic variability is maintained.

In the Epacridaceae, the breeding system of the subfamily Epacrideae appears to be uniformly hermaphroditic. Greater variability is apparent in the Styphelieae, although the majority of species are also hermaphroditic. Complete dioecism, gynodioecism and androgynodioecism have been reported. In some species e.g. *Lissanthe montana*, greater complexity is attained with the occurrence of a "sex strength" system in which the size and pollen content of the anthers vary as well as the pistil development and ovule fertility (Smith-White - 1959a). In *Leucopogon hookeri*, Smith-White has observed male, female and hermaphrodite plants and in *Monotoca scoparia* he records an incomplete dioecious system in which there is a limited seed set in male plants. Both Smith-White (1959a) and Bentham (1869) have recorded complete dioeciousness in coastal populations of *Monotoca elliptica* but Bentham has observed apparently perfect flowers in mountain populations. A gynodioecious breeding system has been reported in *Leucopogon melaleucoides* (McCusker - 1962) and in *Cyathodes douglasii* and *C.tameiameiae* (Sleumer - 1963). Male plants in *C.parvifolia* and *C.divaricata* populations have also been reported (Smith-White - 1955).

Species in Tasmania in which sex differentiation has been observed during this present work include *Cyathodes divaricata, C.juniperina, C.parvifolia, C.*var. *pendulosa, Leucopogon hookeri, Lissanthe montana, Monotoca elliptica, M.empetrifolia, M.glauca, M.linifolia, M.scoparia, M.submutica* and *M.*var. *L.Nicholls*. Except where previously mentioned, the specific breeding system operating in these species is uncertain.

5. Problems of Nomenclature

From an inauspicious beginning, the taxonomy of the family has undergone a continuous series of changes in its nomenclature. The first Epacrid descriptions were made by J.R. and G. Forster in 1776, under the generic name *Epacris* (cited in Copeland - 1954). This name is derived from the Greek words, *epi-* meaning "upon" and *akros*, "the top". It was considered appropriate because the species known at that time were found mostly in alpine habitats.

The first elements of confusion crept into the taxonomy in 1797 when A.J.Cavanilles described several new species. He, too, chose the generic name *Epacris* but the characters ascribed to it were such that J.R. and G. Forster's species were excluded. Cavanilles' genus received recognition and the Forsters' species were subsequently renamed (cited in Copeland - 1954).

With apparent disregard for name priorities, Brown included many name changes in his enlarged treatment of the family in 1810. Von Mueller's partial rejection of Brown's genera and the subsequent inclusion of these into the expanded genus *Styphelia* resulted in further confusion. Not only did it necessitate changes in generic names but also rearrangement and alteration of specific epithets. For example, two species from different genera having a common specific name (such as *Astroloma divaricatum* and *Actrotriche divaricata*) could not both be accomodated in *Styphelia* under their original species name.

As well as von Mueller, many other taxonomists of the time contributed alterations to the nomenclature e.g. Sprengel, Poiret, etc. By 1869 (in Bentham's "Flora Australiensis"), some genera were comprised of species from up to five different sources e.g. *Astroloma* R.Br. included *Venatatia* Cav., *Stenanthera* R.Br., *Stomarrhena* DC., *Pentataphrus* Schlecht. and *Mesotriche* Stschegl.; *Andersonia* R.Br. included *Atherocephala* DC., *Homalostoma* and *Sphincterostoma* Stschegl., and so on. Species with more than three synonyms were not unusual e.g. *Pentachondra pumila* R.Br. was synonymous with *Epacris pumila* Forst., *Styphelia pumila* Spreng., *Leucopogon vaccinoides* Sond., *Pentachondra vaccinoides* Sond., *Trochocarpa pumila* and *Decaspora pumila* F.Muell.; *Cyathodes acerosa* R.Br. (now *C.juniperina* (Forst.) Druce) was the same as *Ardisia acerosa* Gaertn., *Styphelia oxycedrus* Labill., *Cyathodes oxycedrus* R.Br., *Lissanthe acerosa* and *L.oxycedrus* Spreng. Species which have not undergone nomenclature changes are rare and in Tasmania, only 3 species from the subfamily Styphelieae and 12 from the Epacrideae

have no synonyms.

Although the nomenclature in the family has become more stable since 1869, problems have been encountered as recently as 1961. While generally following the scheme of Bentham and Hooker, H.Allan (1961) has reduced the New Zealand species of *Leucopogon* to the genus *Cyathodes*. His reason for this is clearly stated -

> "I have been unable to find any important clear cut differences between those (species) assigned by Cheeseman to *Cyathodes* and those to *Leucopogon*. The characters depended upon to separate the two genera are indicated in the following key: ..... . But several species do not fit at all well into this scheme.".

Notwithstanding the truth of this statement, the changed nomenclature has nevertheless created several problems. These problems would be less significant if only New Zealand species were involved. However, two of the three species of *Leucopogon* involved are also found in Tasmania where, in spite of some intermediate species, there is no difficulty in distinguishing between *Cyathodes* and *Leucopogon*. Hence, if *Leucopogon stuartii* (= *L.fraseri* - New Zealand) and *L.parviflorus* are transferred to *Cyathodes*, then two anomalous species will be present among the Tasmanian representatives of the genus. In addition, two species with confusingly similar names will be placed side by side e.g. *Cyathodes parvifolia* and *C.parviflora*. On the other hand, not to include the two *Leucopogon* species among the Tasmanian *Cyathodes* results in the same species being known by two different names in two countries.

In the context of the whole family, I feel that it would have been more appropriate to leave the nomenclature of the New Zealand species unchanged (i.e. according to Cheeseman - 1906), particularly as the unaltered names must have been in general use.

Confusion in nomenclature has also arisen from Sleumer's work published in 1963 - 64. He proposed the use of von Mueller's scheme and proceeded to either change or revert to the earlier system of nomenclature. Many species involved are largely or wholly restricted to Tasmania in their distribution. With complete disregard for the fact that Bentham and Hooker's system is well established in the state and has been since at least 1903, a whole new series of names were published. The choice between von Mueller's and Bentham and Hooker's classification is largely a matter of convenience, and as such, is

completely dependant on the personal opinion of the taxonomist. The alteration of species names proposed by Sleumer has conferred no advantage over the existing scheme in Tasmania and the outcome, if such changes were generally adopted, would only add to the existing confusion.

Whilst recognising the attempt by Sleumer to rationalize the nomenclature, I feel that he has not appreciated the problems associated with the practical use of his classification. For this reason, I have chosen to ignore the changes suggested by Sluemer and instead, have followed the more familiar and widely used system of Bentham and Hooker. To avoid confusion in this thesis, a list of all species examined and their authorities is given in Appendix C (p. C2).

6. Numerical Size of the Epacridaceae

Without an individual, practical examination of all species, it is virtually impossible to quote an exact figure for the number of species present in the family. An attempt to obtain such a figure from published material involves the integration of many small Floras. In these taxonomic works, different classifications have been adopted, the status of many species and varieties is inconsistent and species with several synonyms are common (see above). Considerable rearrangement and unravelling of nomenclature is required and even then the end result can only represent an approximation of the actual number of species in the family. Nevertheless, I have attempted to do this and as accurately as possible, the species composition of the family is placed at 411. According to Bentham and Hooker's system, these are arranged in two subfamilies and 31 genera. The size of each genus is shown in Table 5 (p. 21). Only three genera *viz. Leucopogon, Dracophyllum* and *Epacris* contain more than thirty species each. Of the remaining 28 genera, 9 are monotypic and 11 others each contain less than ten species.

B. DISTRIBUTION OF THE EPACRIDACEAE

1. General Distribution

Approximately 320 species in the family are confined to Australia (including Tasmania). 95 species are recorded outside Australia, with 48 being found in New Zealand. With one exception, the remaining species are scattered among the islands of the Pacific, Indonesia, New Guinea, the Philippines and Malaya. A single species is found in Tierra del Fuego and Patagonia. The general distribution of the family is shown

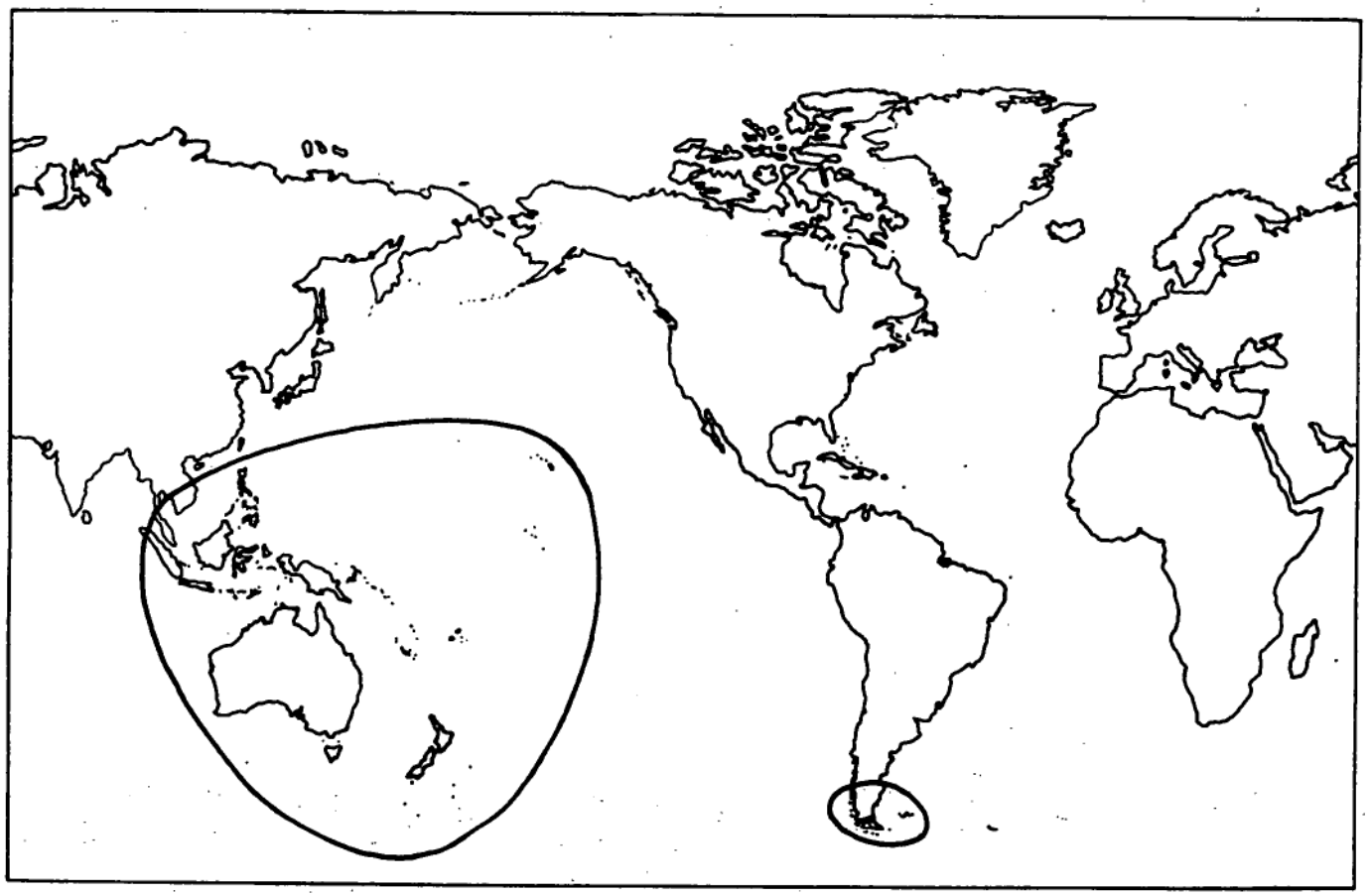


Fig. 3. General distribution of the Epacridaceae.

in Fig. 3 (p. 20), and the distribution of genera is shown in Table 5, below.

Three monotypic endemic genera are not found in Australia. These are *Cyathopsis* (New Caledonia), *Decatoca* (New Guinea) and *Lebetanthus* (South America). Twenty one genera are confined to Australia and the remaining 7 share representation with Australia and with the nearby

Genus	Total	No. of species in W.A.	E.A.	N.Z.	I., P.Is.	S.Am.
Subfamily Epacrideae						
Andersonia	22	22				
Archeria	6		4	2		
Cosmelia	1	1				
Dracophyllum	48		4	35	9	
Epacris	33		31	2		
Lebetanthus	1					1
Lysinema	5	5				
Prionotes	1		1			
Richea	11		11			
Rupicola	2		2			
Sphenotoma	6	6				
Sprengelia	4		4			
Woollsia	1		1			
	141	34	58	39	9	1
Subfamily Styphelieae						
Acrotriche	12	3	11			
Astroloma	18	17	3			
Brachyloma	7	2	5			
Choristemon	1		1			
Coleanthera	3	3				
Conostephium	5	5				
Cyathodes	19		9	4	7	
Cyathopsis	1				1	
Decatoca	1				1	
Leucopogon	159	98	47	4	19	
Lissanthe	3		3			
Melichrus	2		2			
Monotoca	10	3	7			
Needhamia	1	1				
Oligarrhena	1	1				
Pentachondra	3		3	1		
Styphelia	11	4	7			
Trochocarpa	15	1	6		9	
	272	138	104	9	37	0
Total	413	172	162	48	46	1

Table 5. Composition and General Distribution of the Epacridaceae.

(W.A. = Western Australia, E.A. = eastern Australia, N.Z. = New Zealand, I. = Indonesia, P.Is. = Pacific islands including New Caledonia and New Guinea, S.Am. = South America.)

Asian and Pacific regions. These latter genera include *Cyathodes*, *Leucopogon*, *Pentachondra*, *Trochocarpa*, *Archeria*, *Dracophyllum* and *Epacris*. Of these, *Pentachondra*, *Archeria* and *Epacris* are confined to New Zealand. Only 7 species from 4 genera *viz.* *Cyathodes* (1 sp.), *Leucopogon* (4 spp.), *Pentachondra* (1 sp.) and *Trochocarpa* (1 sp.) are common to Australia and the nearby regions, and all are from the subfamily Styphelieae. *Dracophyllum* is the only genus to attain any size outside Australia and has 35 representatives in New Zealand and 9 in New Caledonia.

2. Australian Distribution

Within Australia, the greater number of species is found in the cooler temperate regions (south eastern and south western corners) and the numbers decrease northwards towards the equator. Between the eastern and the western areas of the continent, there is a conspicuous generic and species difference in representation. While this is very obvious in the Epacridaceae, it is by no means confined to the family but is characteristic of the Australian flora as a whole (Hooker - 1860).

Only 7 of the 28 Australian genera are shared by the eastern and western states, and one of these, *Astroloma*, is predominantly Western Australian. All 7 genera are from the subfamily Styphelieae i.e. no genera from the subfamily Epacrideae are common to both areas. Of the remaining 21 genera, 8 are endemic to Western Australia and 13 are endemic to eastern Australia.

The low number of species common to both corners of the continent is even more remarkable than the number of genera. Over half of Western Australia's 172 species are contained in the single genus *Leucopogon* (98 spp.) and only 5 of these are also recorded from eastern Australia. Four other species *viz.* 2 from *Acrotriche* and 2 from *Astroloma* are also shared between the two areas. Hence, a total of 9 species only out of 325 is common to both eastern and Western Australia. The Australian distribution of the family is shown in Table 6 (p. 23).

C. THE EPACRIDACEAE IN TASMANIA

1. Tasmania - Geography and Vegetation

The state of Tasmania is comprised of a major island (Tasmania) and several smaller islands (King, Flinders, Bruni, etc.) lying between 40 and 43½°S latitude (see Fig. C1, Appendix C p. C19). Macquarie Island at 54°S latitude is also included in the state. Tasmania's latitude is matched in the northern hemisphere by Madrid (Spain) and

Genus	Total	W.A.	No. of species in T	V	N.S.W.	Q	S.A.	A.C.T.
Subfamily Epacrideae								
Andersonia	22	22						
Archeria	4		4					
Cosmelia	1	1						
Dracophyllum	4		2		1	1		
Epacris	31		18	8	17	3	1	5
Lysinema	5	5						
Prionotes	1		1					
Richea	11		10	1	1			1
Rupicola	2				2			
Sphenotoma	6	6						
Sprengelia	4		2	1	3	1	1	
Woollsia	1				1	1		
	92	34	37	10	25	6	2	6
Subfamily Styphelieae								
Acrotriche	12	3	1	6	4	2	7	2
Astroloma	18	17	2	3	3		2	1
Brachyloma	7	2	2	4	2	2	3	1
Choristemon	1			1				
Coleanthera	3	3						
Conostephium	5	5						
Cyathodes	9		9	1				
Leucopogon	140	98	9	25	29	19	11	7
Lissanthe	3		2	2	3	1	1	1
Melichrus	2		1	2	2			1
Monotoca	10	3	5	3	3	3	1	1
Needhamia	1	1						
Oligarrhena	1	1						
Pentachondra	3		3	1	1			
Styphelia	11	4	1	2	6	2	1	1
Trochocarpa	7	1	4	1	1	1		
	233	138	38	50	54	32	26	15
Total	325	172	75	60	79	38	28	21

Table 6. Australian distribution of the Epacridaceae.

(W.A. = Western Australia, T = Tasmania, V = Victoria, N.S.W. = New South Wales, Q = Queensland, S.A. = South Australia, A.C.T = Australian Capital Territory.)

Pittsburgh (U.S.A.) but because of its position and small size, it does not suffer the extremes of climate experienced in these two centres.

Tasmania is situated approximately 240 km. south of the south eastern corner of the Australian continent and covers an area of 6.83×10^6 hectares, of which 6.44×10^6 hectares are contributed by

the major island. The land is mountainous and although few peaks exceed 1550 m., their frequency distinguishes Tasmania as one of the most mountainous islands of the world. Rapid changes in soil are apparent, caused by excessive faulting in the parent materials. The soils are mostly acidic and are not considered fertile by world standards (Tasmanian Year Book - 1973, 1974).

The prevailing Westerlies are the dominant influence on the climate which is classified as temperate maritime. The island is large enough, however, to exhibit distinct continental characteristics. The highest and lowest temperatures recorded for the state are 40°C in summer and -12.8°C in winter, but temperatures such as these are restricted to a few days in the year. In summer, the highest mean maxima (c. 24°C) occur at low level inland districts. In winter, coastal areas in the east experience the lowest mean temperatures which are frequently below 3°C. There is a pronounced east-west variation in the climate, particularly rainfall, because of the prevailing Westerlies and the mountainous West Coast topography. The average annual rainfall in coastal areas of the West Coast is 1300 to 1500 mm. but further inland it may reach 3600 mm. In the eastern half of the state, the average rainfall is often less than 700 mm. per year and is as low as 400 to 500 mm. in the rain shadow area of the Midlands. There is no permanent snowline, but snow may remain on the highest peaks until well into summer, and snow showers may be experienced over the highlands at any time during the year.

A mosaic of vegetation patterns are apparent in Tasmania, resulting from the pronounced effects of fire superimposed upon the more usual controlling influences of climate, soil and topography. Much of the vegetation is well adapted to fire and many species, particularly those of the drier areas, are dependent on a high fire frequency for their effective survival. The vegetation may be divided into three broad catagories - austral montane, temperate rainforest and sclerophyll (Jackson - 1965). Austral montane occurs at high altitudes and includes a number of different plant communities which are generally dominated by low shrubs, herbs or sedges. Species from the Epacridaceae are common in these communities. Rainforest occurs in areas where the rainfall exceeds 1400 mm. per year or where the summer average exceeds 50 mm. per month. *Nothofagus cunninghamii* is the dominant species but *Atherosperma, Eucryphia, Phyllocladus* and *Anodopetalum* become increasingly important on the more acid soils. Sclerophyll vegetation

is divided into two types, both dominated by *Eucalyptus* species. Dry sclerophyll is composed of a tall tree layer, a few lesser trees and a medium to low shrub layer. It is generally characteristic of the drier east coast areas. In wet sclerophyll communities, the low shrub layer is absent and has been replaced by a tall shrub layer. Wet sclerophyll is frequently found in the transition zone between dry sclerophyll and rainforest. As well as those described here, other vegetation types occur which cannot be easily placed in any of these categories e.g. sedgeland, coastal heaths, etc., or which are ecotonal between them.

2. The Epacridaceae in Tasmania

The Epacridaceae may be included among the five most widespread and abundant families in the state (see Table 7 below). It contains the highest number of endemics (46) and the second highest number of native species (75). Sixteen genera are represented, 10 from the Styphelieae (39 spp.) and 6 from the Epacrideae (36 spp.). No known introduced species have been recorded, presumably because the Epacridaceae is not found in Europe, Asia or Africa, the origin of most Tasmanian exotics. The family is floristically important but its direct contribution towards the economics of the state is negligible.

		Number of		
Family	Genera	Species	Endemics	Introduced sp.
Epacridaceae	16	75	46	0
Compositae	75	212	37	69
Myrtaceae	8	46	17	0
Leguminosae	35	118	3	55
Proteaceae	12	28	13	0

Table 7. Comparison between the five most widespread families in Tasmania.

A vast range of habitats has been colonized by Epacrid species, from extreme alpine situations to exposed coastal areas and almost all plant communities are supplied with an abundance of species. They vary in size from small shrubs less than 10 cm. in height to tall shrubs or small trees up to 10 m. The plants are woody, rigid and usually prickly. No herbaceous or parasitic species are known although one facultative epiphyte, *Prionotes*, is present in rainforest. The vegetative aspect of two species, *Dracophyllum milliganii* and *Richea*

pandanifolia, closely resembles a monocot habit whilst a third species, *Dracophyllum minimum*, forms a cushion plant which is frequently confused with *Donatia novo-zealandieae* (Donatiaceae) and *Abrotonella forsterioides* (Compositae).

The majority of species are white flowered although some regularly produce pink or red flowers and a few have either cream, yellow, green or orange flowers. No species has blue or purple flowers although the base of the green corolla in *Acrotriche serrulata* flowers has a dark purple band, usually obscured by the sepals. The flowers are small, usually less than 7 - 8 mm. in length but larger in *Prionotes cerinthoides, Astroloma humifusum, A.pinifolium, Styphelia adscendens,* and sometimes in *Epacris impressa.* In several species, the flowers are particularly crowded on the plant forming a conspicuous display, at least to the human eye, and presumably to the appropriate pollinating vectors.

The pollinating agents have not been studied in detail, but insects including the introduced European garden bees, have been observed of the flowers of some species. Ants probably play some role in pollination of the smaller shrubs such as *Acrotriche serrulata, Astroloma humifusum* and *Styphelia adscendens.* Several species, particularly *Leucopogon* species, are strongly scented when in bloom and produce relatively large quantities of nectar.

The fruit of many species from the subfamily Styphelieae are highly coloured either by carotenoids or anthocyanins. Colours range from white, through yellow and orange, to red, blue and purple. The fruit is small (less than 1 cm. in diameter) and may be crowded on the plant. In several species e.g. *Cyathodes*, birds play an important role in seed dispersal and in highland areas, regurgitated pellets containing Epacrid fruits are commonly found. (The bird species involved is the Black Jay, *Strepera fuliginosa*, but others may also be important.) It seems probable that animals may also play some role in dispersal particularly in those species which produce a watery, succulent fruit e.g. *Lissanthe* and *Leucopogon* species.

In the subfamily Epacrideae, a small, inconspicuously coloured capsule is produced which, in many species, is hidden by the sepals. The capsule offers no apparent attraction for seed dispersing agents. The seeds themselves are small and numerous, falling readily once the ripened capsules have opened. They have no attached structures such as wings or hooks to aid in seed dispersal and any role played by

animals and birds is probably quite fortuitous.

D. THE OBJECTIVES OF THIS THESIS

The taxonomy of many genera and species in Tasmania is in an unsatisfactory state, and the family is generally considered a difficult one. A number of species show considerable morphological variation and the presence of several unrecorded species and varieties has led to confusion. The purpose of this thesis is to examine as many of these problems as practicable in the light of chemical information. The compounds chosen for this work were the flavonoids, a widespread class of secondary plant products.

Two alternative approaches were possible i.e. to begin with a known taxonomic problem, examining it thoroughly and expanding outwards to encompass other problems as they became apparent, or to carry out a general survey of flavonoids in the family, enabling trends to be observed and at the same time gaining a superficial knowledge of many taxonomic problems. This latter approach was chosen since it provided a means of comparison with other plant groups as well as enabling any problems encountered to be placed into perspective in the context of the whole family. It became apparent from this work, that three genera in particular were taxonomically "difficult" and it was possible to study these in greater detail.

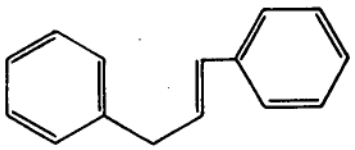
Very little published information involving flavonoids in the Epacridaceae is available. Gascoigne *et al.* (1948) reported the occurrence of anthocyanin derivatives in 15 species but the sugars involved in glycosylation were not determined. In 1962, Bate-Smith investigated 3 species for the accumulation of cyanidin, delphinidin, kaempferol, quercetin and myricetin and in 1973, the distribution of the latter three aglycones in 27 species was reported by Harborne and Williams.

PART II

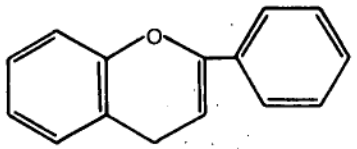
EXPERIMENTAL SECTION

A. INTRODUCTION TO FLAVONOID COMPOUNDS

Flavonoid compounds form the largest group of naturally occurring plant phenols (Siekel - 1964) and include those compounds containing two aromatic rings linked by three carbon atoms i.e. having a C_6-C_3-C_6 structure (I). In the more common flavonoids, the central C_3 portion is joined with an oxygen atom to form a heterocyclic ring (II).



I



II

Anthocyanins, flavones, dihydroflavones, isoflavones, catechins, leucoanthocyanidins, biflavonyls, chalcones, dihydrochalcones and aurones are included among the flavonoids. A range of flavonoid structures and their numbering system is shown in Fig.4 (p.29). Structural variability within each group is derived from hydroxylation, methoxylation or methylation of different carbon atoms in the molecule. In addition, hydroxyl groups or carbon atoms may have various glycosidic groups attached. In rare instances, aliphatic or non-organic groups e.g. SO_3K^- may be present.

1. Relevance to Systematics

The advantages of using flavonoid compounds in phytochemical work include their relative stability combined with structural variability, ease of detection and scoring, and relatively simple purification and identification techniques. As secondary plant products, slight structural modifications are less likely to be detrimental than those occurring among the primary products, where small changes may critically effect plant metabolism. Hence, a range of structural types may be maintained and by carefully assessing their distribution it may be possible to confirm (or otherwise) relationships already suggested on the basis of alternative data such as morphology, cytology, etc.

The relevance of flavonoids to this type of work is becoming

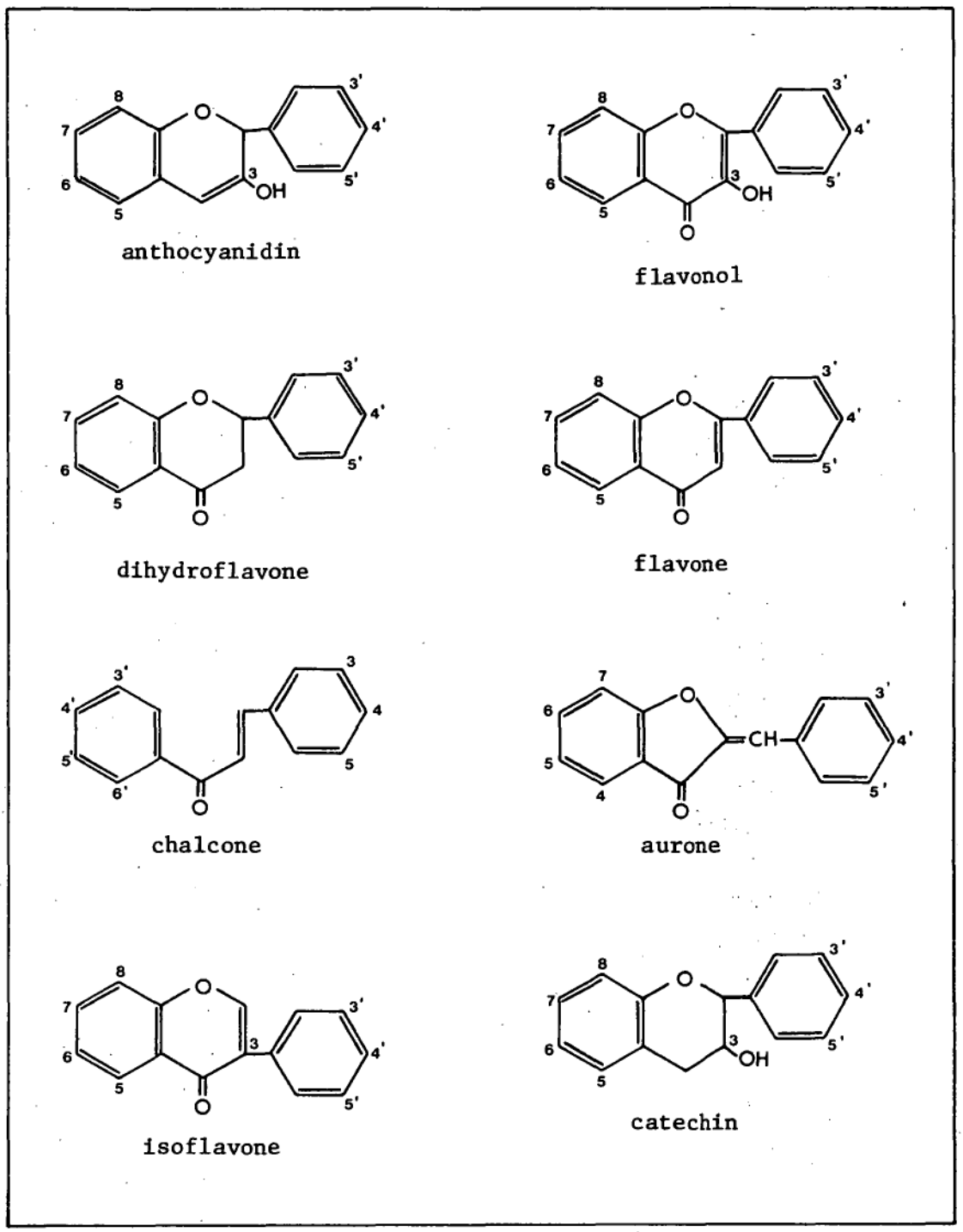


Fig. 4. Stucture and numbering system of some flavonoid compounds.

increasingly apparent as more data becomes available from plant surveys e.g. Bate-Smith (1962, 1968), Markham, Mabry and Swift (1970), Harborne and Williams (1971a, 1973), Williams and Murray (1972), etc. The level at which significant contributions are made varies in the different plant groups. It may occur at, or above, the family level e.g. Moore, Harborne and Williams (1970), Glennie and Harborne (1971), Williams, Harborne and Clifford (1971), etc.; at the subfamily or tribal level e.g. Crowden, Harborne and Heywood (1969), Harborne and Williams (1972), etc.; at the generic or subgeneric level e.g. Harborne, Heywood and Saleh (1970), Natarajan *et al.* (1970), etc. or at, or below, the species level e.g. McClure and Alston (1966), Arditti (1969), Valdes (1970), Markham (1973), etc.

Flavonoids have also been used in studies of hybridization and in attempts to determine ancestry e.g. Turner and Alston (1959), Harney and Grant (1964), McHale and Alston (1964), Bridle *et al.* (1973), etc.

The distribution of flavonoid compounds has been examined by Harborne (1967) and although the amount of published data has increased considerably since this work, the general pattern of distribution has changed very little. In the following text, specific references have been given only where some modification of the distribution pattern has occurred since 1967.

Within the plant kingdom, flavonoid studies have indicated a progressive trend towards greater complexity in structure from the lower groups through to the Angiosperms. Only a few flavonoids including O- and C-glycosylflavones and 3-deoxyanthocyanins are known in the Bryophytes but the range is increased in the Pteridophytes and Gymnosperms and representatives of most classes have been found. In the Pteridophytes, biflavones (Okigawa *et al.* - 1971), anthocyanins, 3-deoxyanthocyanins, flavonols, O- and C-glycosylflavones, chalcones, dihydrochalcones, dihydroflavones and leucoanthocyanidins have been reported. With the exception of chalcones, these have also been reported in the Gymnopserms where the biflavonyls, rare in the Pteridophytes, are relatively widespread. An additional class, the isoflavones, are also present but are uncommon. In the Angiosperms, all flavonoid types have been found and structural complexity is greater than in the lower groups. However, some classes of compound are very restricted in distribution e.g. 3-deoxyanthocyanins (mainly in the Gesneriaceae), isoflavones (mainly in the Leguminosae) and biflavonyls (sporadic occurrence in the Casuarinaceae, Caprifoliaceae, Anacardiaceae,

etc.).

Because the production of chemical compounds occurs in a logical sequence of biosynthetic steps, the direction in which structural changes has occurred in a series of related chemical types can be predicted, and is usually less ambiguous than the direction of changes occurring in morphological characters. With the latter characters, it is frequently possible to show progressive changes but these may be read in more than one direction since they cannot, as yet, be related to a more or less fixed pathway. However, if they are accompanied by a logical progression in chemical characters, the direction of evolution in morphological characters may be indicated by the direction of the accompanying chemical changes. In turn, the presence of a morphological sequence supports the construction of a chemical evolutionary scale.

On the basis of their occurrence in the plant kingdom, Harborne (1967) has proposed a possible evolutionary scale for some flavonoid compounds. Probable "advanced" characters include complex O-glycosylation, 2'-hydroxylation, trihydroxylation of the anthocyanidin B-ring (in flowers), elimination of leucoanthocyanidins, replacement of flavonols by flavones (in leaves) and the oxidation of chalcones to aurones. Probable "primitive" characters include the presence of 3-deoxy-anthocyanidins, leucoanthocyanidins, chalcones, flavanones, dihydrochalcones and C-substitution. A trend in hydroxylation, from 8-hydroxyflavonols (primitive) through 8-hydroxyflavones, 6,8-dihydroxy-flavones and 6-hydroxyflavonols (intermediate) to 6-hydroxyflavones (advanced) is also suggested (Harborne and Williams - 1971a).

While it is relatively easy to demonstrate the potential of flavonoids in taxonomic studies, it must be emphasized that chemical characters cannot generally be considered of greater or lesser importance than other character types until scientific research has proved this unequivocally. Evolution of characters in morphology, cytology, chemistry, anatomy, etc. are not necessarily correlated and may occur at different rates and even in apparently opposite directions. Where conflicting results occur between chemical and morphological, or other types of data, extreme care must be used before making dogmatic taxonomic pronouncements on the basis of chemical evidence. These characters cannot be excluded from the same processes of convergence, parallelism, selection, etc., which operate on other types of characters. Ideally, taxonomic interpretations should be based on several lines of

evidence and where this is so, the resulting deductions must be considered potentially more valid than those based on any single source of information.

2. Function and Biosynthesis

Many aspects of the function and biosynthesis of flavonoids are still open to speculation. The basic C_{15} molecule has never been isolated from plants but is known to be derived from the condensation of two malonyl Co-A units with one acetyl Co-A unit (A-ring) combined with a cinnamic acid residue obtained via the shikimic acid pathway (B-ring and central C_3). All flavonoid classes are derived directly or indirectly from this C_{15} unit.

The stage at which hydroxylation occurs in the flavonoid molecule is not clear. Evidence suggests that B-ring hydroxylation may occur either before C_9 or after the C_{15} stage but hydroxylation at other positions, excluding positions 5 and 7, probably occurs after it. Methylation and glycosylation appear to be terminal reactions.

A possible biosynthetic pathway, using biologically feasible steps and in no way conflicting with available evidence has been proposed by Harborne (1967). Research carried out since 1967 has not substantially altered this scheme which is outlined in Fig. 5 (p. 33).

Along with carotenoids and chlorophylls, flavonoids are responsible for most pigmentation in plants. It is not clear whether this is their primary function, especially in view of their widespread distribution, but their importance in this respect is difficult to dispute. Different pollinating agents (birds, insects, etc.) may be sensitive to particular flower colours, and within certain plant species, there may be selection towards the appropriate pigments. Some flavonoids reflect light from the UV region of the spectrum and consequently attract pollinators sensitive to this form of light.

Pigmentation may also be important as an attractant in seed dispersal or even in germination, if the seeds have some requirement, either facultative or obligative, to pass through a digestive tract. The taste of flavonoids would be expected to have some importance in this respect and also in determining the plant species preferred by browsing animals and insects. In man, the taste sensitivity to these compounds is very poor and only a few dihydrochalcones (bitter), leucoanthocyanidins (astringent) and some chalcones and dihydrochalcones

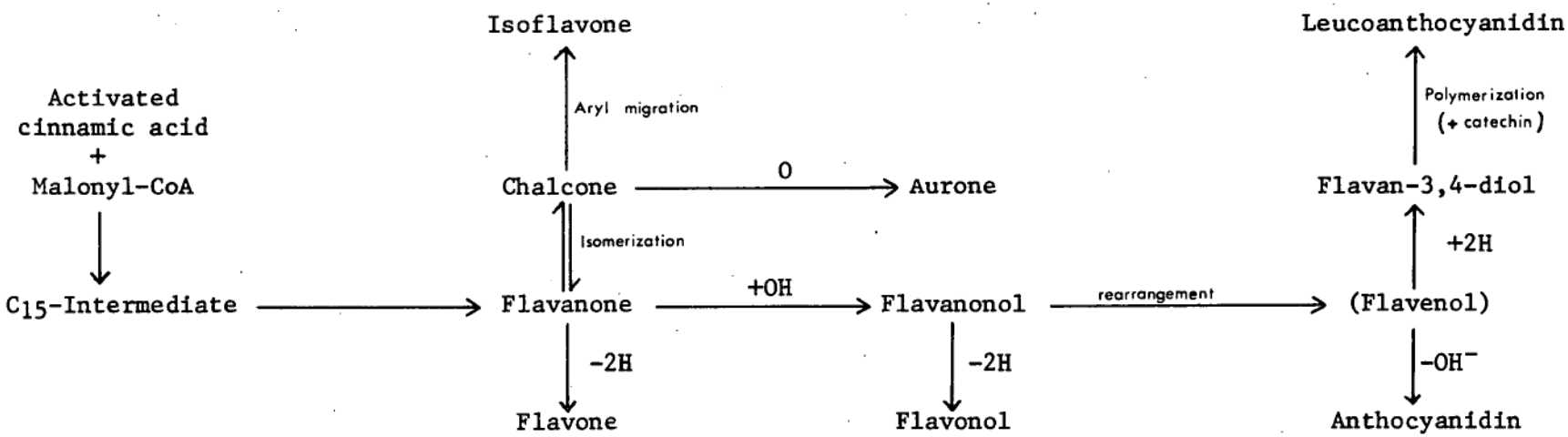


Fig. 5. Biosynthetic pathway of the flavonoids (taken from Harborne - 1967).

(sweet) are recognized to contribute significantly to flavour. The taste sensitivity of birds and animals feeding on tissues with a high flavonoid content is not known but it has been reported recently that some insects are effected by the presence of these compounds (Zielske *et al.* - 1972, Doskotch *et al.* - 1973).

3. Identification Procedures

Much of the work involved in purifying and identifying flavonoid compounds is based on chromatography (paper, column or thin layer). This technique makes use of the differential mobility of compounds in various solvent systems and on different adsorbents. UV spectroscopy is also used routinely and is particularly important with flavonoids other than anthocyanins. Individual compounds have characteristic spectra and the height, position and number of absorption bands contributes information about structural features. Spectra are usually measured in MeOH (less commonly EtOH) or MeOH/HCl (for anthocyanins and 3-deoxyanthocyanins) before and after hydrolysis and with the addition of various reagents such as $AlCl_3$, NaOMe, etc. (see Appendix A). Compounds may be acetylated or methylated and the spectra compared before and after these procedures.

Hydrolysis procedures are used to remove the sugar from the aglycone. Mineral acid is used to completely detach the sugar(s), organic acid to remove di- or tri-saccharides intact, and alkaline hydrogen peroxide to remove di- and tri-saccharides intact from position 3 of the molecule (or from position 7 in some dihydroflavones). Alkaline hydrolyses may be used to remove the acylating groups from the sugar moiety, when present. Enzyme hydrolyses are used to determine the type of linkage between the aglycone and the sugar, and to confirm the identity of the latter. In all hydrolyses, the rate is important in suggesting the identity of the sugar and the linkage involved.

Degradative procedures such as reductive cleavage, are used to split the molecule, and subsequent analysis of fragments may indicate the structure of the A and B rings.

Other techniques are also used in flavonoid identification e.g. nuclear magnetic resonance spectroscopy, mass and infra red spectroscopy, gas chromatography, etc. These techniques require expensive and sophisticated equipment, and considerable experience in interpreting data. None of these methods has been used in this present research. In this laboratory, techniques were centred around chromatographic,

hydrolytic and spectral procedures. These have definite limitations when dealing with complex, novel flavonoids, but they are adequate to purify and identify the simpler compounds.

4. Sugars involved in Glycosylation

Nine monosaccharides (8 aldoses and 1 ketose) are involved in glycosylation in flavonoids, but not all have been found in each class of compound. One ketohexose (fructose), 2 aldohexoses (glucose, galactose), 2 uronic acids (glucuronic and galacturonic acid) and 4 aldopentoses including arabinose, xylose, one methyl pentose (rhamnose) and one rare branched pentose (apiose) have been detected. All are D sugars with the exception of rhamnose and arabinose which have only been found in the L form. The sugars may be joined to the aglycone with either an α- or β-link but in flavonoids whose configuration has been determined absolutely, D sugars are usually β-linked and L sugars are commonly α-linked. Arabinose is unusual in forming either an α- or β-link, but the former is the more common. Arabinose may also occur in either a pyranose of furanose form.

The most common sugar involved in glycosylation is glucose. It is known in all classes of flavonoids, and is usually the only sugar present in the smaller groups such as chalcones, aurones, dihydrochalcones, etc. In anthocyanins, monoside derivatives containing glucose, galactose, rhamnose, arabinose and fructose are known but xylose, apiose and the two uronic acids have not been reported as yet. Fructose is extremely rare and has only been reported in *Salix* (Bridle *et al.* - 1973) and *Mentha* (Shakova - 1971). However, in some cases, its presence may have remained undetected since the normal identification procedures (hot mineral acid) would be severe enough to cause its conversion to glucose. All sugars have been found in monoglycosylated flavones and flavonols excepting apiose(which is known in biosides), and fructose.

More than one sugar molecule may be attached to the flavonoid nucleus, either at separate positions or at the one position as a disaccharide, or less frequently, a trisaccharide. The configuration of some of the more common disaccharides is shown in Table 8 (p. 36).

Trisaccharides are rare in flavonoid compounds but have been found in both flavonols and anthocyanins. The sugars may be branched as in 2^G-glucosylrutinose or in a linear arrangement as in gentiotriose.

Acylating groups, ususally p-coumaric and ferulic acid, are sometimes attached to sugar units at position 3 of the flavonoid

molecule. Acids such as gallic (El Sissi *et al.* - 1973), malonic (e.g. Bloom and Geissman - 1973, Kreuzaler and Hahlbrock - 1973) and acetic (e.g. Fong *et al.* - 1973, Anderson *et al.* - 1970) have been reported infrequently.

Trivial name	Structure
rutinose	6-O-α-L-rhamnosyl-D-glucose
robinobiose	6-O-α-L-rhamnosyl-D-galactose
gentiobiose	6-O-β-D-glucosyl-D-glucose
sophorose	2-O-β-D-glucosyl-D-glucose
sambubiose	2-O-β-D-xylosyl-D-glucose
lathyrose	2-O-β-D-xylosyl-D-galactose
neohesperidose	2-O-α-L-rhamnosyl-D-glucose

Table 8. Structure and common name of some flavonoid disaccharides.

Two flavonoid classes, anthocyanins and flavonols, are widespread in the Epacridaceae, and although a few representatives of the minor classes are present e.g. chalcones, aurones, dihydroflavones, etc., their distribution is very restricted. In this research, chemical experimentation was mainly centred on anthocyanins and flavonols, and for this reason, discussion in the following text will be essentially limited to these two classes.

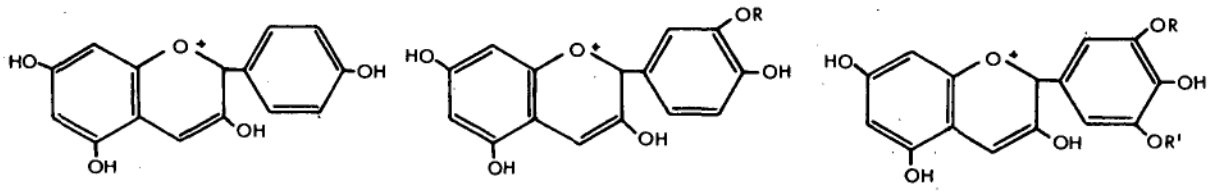
5. Anthocyanins

2-Phenylbenzopyrylium (flavylium) salts are known as anthocyanins (Fig. 4, p. 29). They are coloured compounds whose glycosides are responsible for most blue, purple, red, pink and orange colours found in the plant kingdom.

There are six common anthocyanidins and each is hydroxylated at positions 3, 5, 7 and 4'. In addition, one or two hydroxy or methoxy groups may be present in the B-ring (Fig. 6, p. 37). A few rare anthocyanidins are known in which the 5 or 7 positions are methylated (e.g. hirsutinidin, rosinidin, capsinidin) or in which the 6 or 8 position is hydroxylated (e.g. aurantinidin). In addition, an unusual series of anthocyanidins have been found in which the 3-hydroxyl group is entirely lacking (3-deoxyanthocyanidins).

Colour properties of anthocyanidins are related to the hydroxylation patterns of the B-ring e.g. in acidic methanolic solution, pelargonidin appears orange-red, cyanidin appears pink-red and delphinidin appears purple-red. The effect of methylation on the B-ring is barely

detectable in solution but on paper, methylated anthocyanidins appear slightly more pink than their non methylated analogues.



pelargonidin

cyanidin (R = H)
peonidin (R = Me)

delphinidin (R = R' = H)
petunidin (R = Me, R' = H)
malvidin (R = R' = Me)

Fig. 6. Structures of the six common anthocyanidins.

When subjected to spectral analyses, anthocyanidins exhibit two main absorption peaks, one in the visible region between 520 and 550 nm. (Band I) and the other in the UV region between 270 and 277 nm. (Band II). Increased hydroxylation in the B-ring results in a marked bathochromic shift of Band I but methylation causes only a very slight hypsochromic shift e.g. pelargonidin - 520, 270 nm., cyanidin - 535, 277 nm., delphinidin - 546, 277 nm., petunidin - 543, 276 nm., malvidin - 542, 275 nm. (Harborne - 1967).

Anthocyanins are thought to occur naturally only as glycosides (anthocyanins) since the aglycone itself (anthocyanidin) is unstable in aqueous solution and is relatively insoluble compared to the glycoside. However, two reports of the occurrence of free anthocyanidins have been published recently (Mullick - 1969, Lowry - 1971a). Anthocyanins occur in the cell sap, probably in association with any one of several anions derived from organic acids. The identity of the actual anion present *in vivo* is not known because anthocyanins are normally extracted as chlorides (using MeOH/HCl). The use of neutral or alkaline extracting solutions is not favoured because under these conditions, anthocyanins are converted respectively to colourless pseudo-bases, or coloured quinonoid compounds called anhydro-bases (Dean - 1963). Under the usual system of nomenclature, the anion is ignored but its presence is assumed.

In naturally occurring anthocyanins (excepting 3-deoxyanthocyanins) at least one sugar is always present at position 3 but additional sugars may also be present at this position or at either positions 5 or 7. A few 3-hydroxy anthocyanins with sugars only at positions 5 or 7 have been synthesized. Anthocyanins with sugar substituents in the B-ring

are unknown. With 3-deoxyanthocyanins, position 3 is not available for substitution and sugars are attached at positions 5 and/or 7.

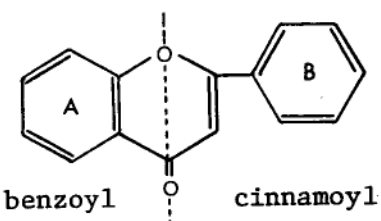
Anthocyanins are generally thought to occur as single units but recent evidence (Bridle *et al.* - 1973) suggests that dimers may sometimes occur. The actual position through which the two molecules are linked has not been established unambiguously although position 4 is thought to be involved.

6. Flavones and Flavonols

Flavones (2-phenylbenzopyrones) may be divided into two groups depending on the presence or absence of a hydroxyl substituent at position 3. Although this division is one of convenience because of the large number of structures known, 3-hydroxy flavones (flavonols) can usually be readily separated from flavones by spectral, colour and chromatographic properties. A further difference between the two is apparent in their distribution, at least among the Angiosperms where surveys of leaf pigments have indicated that flavonols tend to be associated with woody plants whereas flavones are associated more with herbaceous plants (Bate-Smith - 1962, 1968). This distribution has some phylogenetic significance in that the progression from highly lignified (woody) tissues to herbaceous tissues is considered as a trend towards greater advancement (Hutchinson - 1973).

In spite of their widespread occurrence in the plant kingdom, no specific unambiguous function has been assigned to flavones and flavonols apart from their role in pigmentation. They are important as yellow pigments and they may be present in white organs where they add "body" to tissues that would otherwise be translucent (Harborne - 1967). They reflect light in the UV region of the spectrum and hence, may play some part in attracting animal vectors sensitive to this form of light. In addition, they may be involved in co-pigmentation (e.g. Nozzolillo - 1970, Asen *et al.* - 1972).

In methanol, flavones and flavonols exhibit two main absorption peaks (Band I and II) between the wavelengths 240 and 400 nm. Band I (300 - 380 nm.) is chiefly associated with the B-ring cinnamoyl system and Band II (240 - 280 nm.) with the A-ring benzoyl grouping.



The intensity and position of these bands is influenced by the number of electron donating groups e.g. hydroxyl and methoxyl groups, in each ring. These alter the relative resonance contributions of either ring with respect to the total resonance contribution of the whole molecule e.g. the introduction of hydroxyl groups into the B-ring but not into the A-ring causes a large bathochromic shift of band I and an increase in intensity. A similar effect is seen in Band II when A-ring oxygenation is increased (Jurd - 1962, Mabry *et al.* - 1970).

The position of the Band I peak in methanol differs between the common flavones and flavonols, occurring between 304 and 350 nm. in flavones, 328 and 357 nm. for 3-substituted flavonols and between 352 and 385 nm. in flavonols with a free 3-hydroxyl group (Mabry *et al.* - 1970). Additional information may be obtained from the use of specific reagents such as NaOMe, $AlCl_3$, $AlCl_3$/HCl, NaOAc and H_3BO_3, and the magnitude of the spectral shifts may be correlated with structural characters (see Jurd - 1962, Mabry *et al.* - 1970).

The number of hydroxyl groups may vary but in general, as the overall state of oxygenation increases in the molecule, so does the frequency of 3-hydroxylation. Hence, pigments with less than four hydroxyl groups are usually flavones whilst those with six or seven are usually flavonols (Dean - 1963). Compounds such as 5,7-dihydroxy-flavonol (galangin) in which only one ring is hydroxylated, are rare among flavonols.

Three flavonols are particularly common throughout the plant kingdom i.e. kaempferol (3,5,7,4'-tetrahydroxyflavone), quercetin (3,5,7,3',4'-pentahydroxyflavone) and myricetin (3,5,7,3',4',5'-hexahydroxyflavone). 6-, 8- and 2'-hydroxyflavonols and 5-deoxyflavonols are known but are rare in occurrence. Methoxylated flavonols e.g. rhamnetin, azealetin, syringetin, etc., have been isolated but isorhamnetin (3'-methylquercetin) is the only one of common occurrence.

Flavonols occur in plant tissues as glycosides with the sugar units attached most frequently at position 3. However, substitution may also occur at positions 7, 4', 3' and 5, although the latter is particularly rare (Glennie and Harborne - 1971). This position is thought to be protected from glycosylation by hydrogen bonding with the adjacent carbonyl group (Harborne - 1967). For this reason, when a second position (apart from position 3) is involved in glycosylation, position 7 rather than 5 is usually substituted. Thus, in flavonols, 3,7-diglycosides correspond with 3,5-diglycosides in anthocyanins.

On paper chromatograms, flavonols appear as dark absorbing spots which change to various characteristic shades of yellow when fumed with ammonia. Thus, kaempferol glycosides appear yellow-green with NH_3, quercetin glycosides appear yellow and myricetin glycosides appear yellow- brown . Their mobility in organic solvents such as BAW is higher than for the analagous anthocyanins but is approximately the same in aqueous solvents. The rate of hydrolysis of flavonol glycosides is faster than for anthocyanins and may be correlated with the identity of the sugar and its position of attachment i.e. sugars at position 3 are detached in the order rhamnose = arabinose > galactose = glucose > glucuronic acid. Compounds with sugars at position 7 are particularly resistent to hydrolysis.

B. RESULTS

In the following text, the term "dominant" will be used in connection with the relative concentrations between pigments. Its use here is completely dissociated from any genetical connotations it may have. The term "flavonoid", with inverted commas, is used to include all flavonoid compounds excepting anthocyanins.

Abbreviations used for pigment structures are given below, and those used for solvents, reagents and sprays are given in Table A1 (Appendix A - p. A13).

Anthocyanidins

Pel = pelargonidin
Cy = cyanidin
Dp = delphinidin
Mv = malvidin
Pet = petunidin

Flavonols

Km = kaempferol
Qu = quercetin
My = myricetin
Is = isorhamnetin

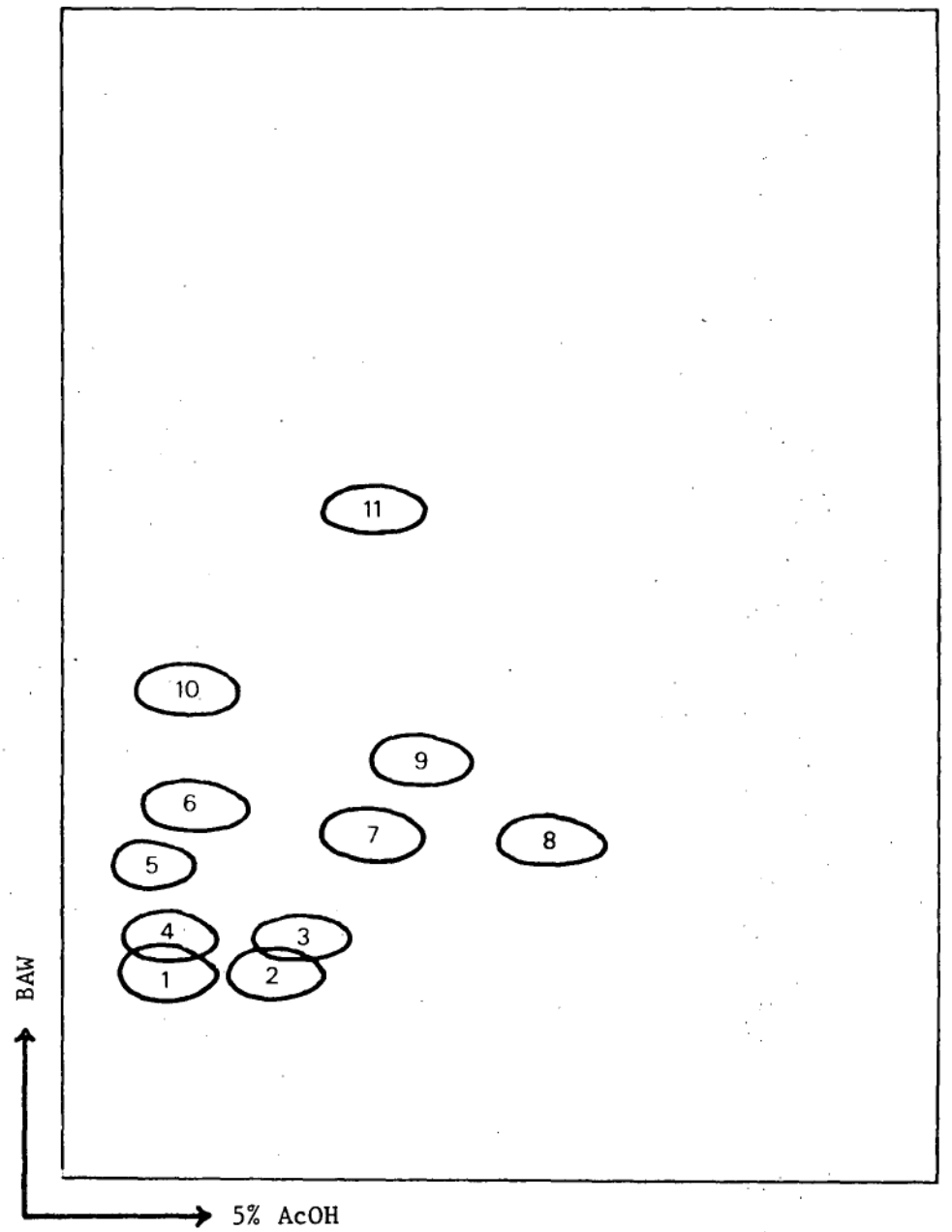
Sugars

Arab = arabinose
Glc = glucose
Gal = galactose
Glur = glucuronic acid
Rha = rhamnose
Xyl = xylose

Over 60 different flavonoid compounds were detected in the Epacridaceae and the identity of 27 was determined unambiguously.

1. Identification of Anthocyanins

Twenty one anthocyanins were observed in the family including 8 cyanidin, 6 delphinidin, 5 pelargonidin and 2 malvidin derivatives. These include 12 monosides and 9 biosides. Pigments identified unambiguously include Cy-3-arabinoside, Cy-3-galactoside, Cy-3-glucoside, Cy-3-rhamnoside, Cy-3-rhamnosylgalactoside, Cy-3-rhamnosylglucoside, Cy-3-xylosylarabinoside, Cy-3-xylosylgalactoside, Dp-3-arabinoside, Dp-3-galactoside, Dp-3-rhamnosylgalactoside, Dp-3-rhamnosylglucoside, Pel-3-glucoside and Pel-3-rhamnosylglucoside.



LEGEND

1 = Dp-3-Gal	6 = Cy-3-Gal (≃ Cy-3-Glc)
2 = Dp-3-RhaGal	7 = Cy-3-RhaGal (≃ Cy-3-RhaGlc)
3 = Dp-3-RhaGlc	8 = Cy-3-XylGal
4 = Dp-3-Glc	9 = Cy-3-XylArab
5 = Dp-3-Arab	10 = Cy-3-Arab
	11 = Cy-3-Rha

In visible light, pigments 1 - 5 = purple, 6 - 11 = pink.

Fig. 7. Chromatographic position of major Epacrid anthocyanins.

The identification of 7 pigments is tentative only i.e. Dp-3-glucoside, Dp-3-xylosylgalactoside, Pel-3-arabinoside, Pel-3-galactoside, Pel-3-rhamnosylgalactoside, Mv-3-arabinoside and Mv-3-galactoside.

The chromatographic position of the major Epacrid anthocyanins is shown diagrammatically in Fig. 7 (p. 42), and R_f values are given in Table A3 (Appendix A, p. A15).

Four pigments (Cy-3-Arab, Cy-3-Gal, Cy-3-RhaGlc and Dp-3-RhaGlc) were first identified during 1969, and the work was included in an Honours thesis. The identifications were subsequently confirmed during this present research.

Comments on Pigment Identifications

(a) Cyanidin 3-xylosylarabinoside (Cy-3-epacroside)

The chromatographic behaviour of Cy-3-XylArab (see Fig. 7) was sufficiently different from any published data to suggest immediately that its glycosidic substituent might be unusual. Acid hydrolysis and treatment with alkaline hydrogen peroxide indicated that the sugars in this anthocyanin were present as a disaccharide containing xylose and arabinose. The ratio of the colour intensity of the two sugars, after spraying chromatograms with aniline hydrogen phthalate, was visually estimated as 1:1. Spectral data confirmed that no substituent was present at position 5, and spectral and chromatographic behaviour indicated that the pigment was not acylated. A partial hydrolysis, with subsequent isolation and identification of Cy-3-Arab as the intermediate, indicated the order in which the sugars were attached to the aglycone. The yield of cyanidin 3-monoside from the partial hydrolysis was small compared with that obtained from other 3-biosides isolated from the Epacridaceae, suggesting that the aglycone-sugar link is unusually labile under acid conditions.

Cyanidin 3-epacroside was detected in only 2 of the 85 species examined during the survey. It occurred as a minor pigment and its presence in each species was not consistent. The disaccharide in this anthocyanin is of particular interest since it has not been reported previously as a substituent in any class of flavonoid. However, xylosylarabinosides are known to occur naturally as components of polysaccharides e.g. in corn hull and barley husk hemicelluloses, and in apple gum (Montgomery *et al.* - 1957, Aspinall and Ferrier - 1957, Charlson *et al.* - 1957).

(b) Cy-, Dp- and Pel-3-rhamnosylgalactoside

The chromatographic behaviour of Cy- and Dp-3-RhaGal was very

similar to that of Cy- and Dp-3-RhaGlc respectively, suggesting that the configuration of this disaccharide is analagous to rutinose (1,6-α-rhamnosyl-D-glucose). Prior to its detection in the Epacridaceae, a disaccharide of this type (1,6-α-rhamnosyl-D-galactose, robinobiose) had not been reported in anthocyanins although it is known as a constituent of several flavonols i.e. Km-, Qu-, My- and Is-3-robinobioside 7-rhamnoside (Harborne - 1967). Since details of these anthocyanins were published (Jarman and Crowden - 1973) two anthocyanins containing robinobiose (Pel- and Cy-3-robinobioside) have been reported in the Cornaceae (Du and Francis - 1973). The chromatographic data published for Cy-3-robinobioside from *Cornus* compares favourably with that obtained from the Epacrid anthocyanin and it seems probable that their structure is the same.

A minor orange coloured anthocyanin, tentatively identified as Pel-3-RhaGal, was not hydrolysed but its colour and chromatographic behaviour was consistent with those expected from an anthocyanin of this structure. (A chromatographic comparison with Pel-3-RhaGlc indicated that the two pigments were similar in structure although not identical.)

(c) Cy- and Dp-3-xylosylgalactoside

From a comparison of the chromatographic behaviour of the parent anthocyanins, it seems probable that the sugar in Cy-3-XylGal is structurally analagous to sambubiose (1,2-β-xylosyl-D-glucose). Lathyrose (1,2-β-xylosyl-D-galactose) is rare in anthocyanins and has been reported only from the Leguminosae (Harborne - 1967) and the Araliaceae (Sakamura and Kawano - 1970).

A minor delphinidin 3-bioside was also isolated, but because of insufficient purified material, it was not possible to determine the sugars unambiguously. The R_f of the intermediate product obtained during partial hydrolysis was similar to that of Dp-3-Gal, but complete hydrolysis of the contaminated pigment yielded glucose as well as xylose and galactose. The pigment has been tentatively identified as Dp-3-xylosylgalactoside on the basis of these hydrolysis results and also because of its co-occurrence with Cy-3-XylGal and parallel chromatographic behaviour.

(d) Dp-3-arabinoside

Dp-3-Arab is a novel anthocyanin whose isolation had not been reported prior to this study although its presence in mixture with other

anthocyanidin 3-arabinosides in berries of *Vaccinium angustifolium* (Ericaceae) had been reported (Francis *et al.*- 1966). In the Epacridaceae, it occurred as a dominant pigment only in the twig bark.

(e) Pel-3-arabinoside and Pel-3-galactoside

These two pigments were trace constituents and were only tentatively identified. Their colour and chromatographic behaviour in comparison with Pel-3-Glc (and Pel-3-RhaGlc) was consistent with their proposed structures. Their co-occurrence with Cy-3-Arab and Cy-3-Gal also supported their identification.

(f) Mv-3-arabinoside and Mv-3-galactoside

These pigments were detected in only one species (*Woollsia pungens*) endemic to eastern Australia but not present in Tasmania. This species commonly has white flowers but in certain locations e.g. Lawson and Helensburgh (N.S.W.), the petals may be various shades of purple-pink.

The two anthocyanins were not readily separated from cyanidin contaminants and significant quantities of material were lost during the purification process. Even after removal of cyanidin derivatives, some flavonol contamination remained (probably My-3-Arab). Almost certainly, this could have been removed using a polyamide column (see Appendix A - p. A7-A8) but any further loss of material, however small, would have been crucial.

The two pigments contained the same aglycone which occupied the appropriate R_f position for malvidin on Forestal and Formic Acid chromatograms. This identification was confirmed from a spectral examination of the unhydrolysed pigment i.e. the OD_{max} (in MeOH/HCl) occurred at 538 nm. and was unaffected by the addition of $AlCl_3$. (The presence of contamination did not seem to interfere with the spectra in the visible region.) Both galactose and arabinose were present among the hydrolysis products from each pigment but some cross contamination between pigments was apparent. In addition, some arabinose is thought to have been contributed by My-3-Arab, the suspected impurity. Hence, the two pigments are tentatively identified as Mv-3-Gal and Mv-3-Arab. The latter pigment, Mv-3-Arab, has recently been reported in the Lythraceae along with Pet-3-Arab and Dp-3-Arab (Saleh - 1973).

(g) Anthocyanidin galactosides and glucosides

The identity of anthocyanins from two glycosidic series *viz.* anthocyanidin galactosides and anthocyanidin glucosides, was not proven unequivocally in all species from which they are reported in Table A4

(p. A16). Comparable pigments from either series i.e. Cy-3-Gal - Cy-3-Glc, Cy-3-RhaGal - Cy-3-RhaGlc, Cy-3-XylGal - Cy-XylGlc, Dp-3-Gal - Dp-3-Glc and Dp-3-RhaGal - Dp-3-RhaGlc, showed similar chromatographic behaviour and could not be differentiated on routine 1- or 2-D paper chromatograms. Co-chromatography was successful only when purified pigments were compared under identical conditions and at equivalent concentrations. Analysis of hydrolysis products readily indicated which sugar was present but the use of this method was impractical for the large number of samples containing the "problem" anthocyanins. Hence, the identity of anthocyanins in many species was assumed using indirect evidence i.e. within the family, the hydrolysis results of 22 species indicated that

(a) anthocyanidin galactosides and glucosides are mutually exclusive and

(b) anthocyanidin galactosides are common (20/22 spp.) whilst the glucosides are rare (2/22 spp. - from one genus only).

Thus, on the basis of this information, the identity of non hydrolysed anthocyanins from a number of species has been predicted. With exceptions in 2 species only, pigments that chromatographed favourably against the appropriate anthocyanidin galactoside, or appeared in the "correct" position on 2-D chromatograms have been assumed to contain galactose rather than glucose. Pigments from *Trochocarpa gunnii* and *T.disticha* are believed to contain glucose rather than galactose. This follows from the detection of Cy- and Dp-3-RhaGlc as major constituents in the fruit of both species and the occurrence in *T.gunnii*, of Cy-3-Glc as a minor constituent in the fruit and a major constituent in the twig bark.

Species from which anthocyanins were hydrolysed for the detection of galactose and glucose include *Epacris gunnii* (twig bark), *E.impressa* (flowers; twig bark), *Prionotes cerinthoides* (flowers), *Richea pandanifolia* (immature capsules), *Woollsia pungens* (flowers; sepals and leaves), *Acrotriche divaricata* (fruit), *Astroloma humifusum* (flowers), *Cyathodes dealbata* (fruit), *C.divaricata* (fruit), *C.glauca* (fruit), *C.nitida* (fruit), *C.straminea* (fruit), *Leucopogon ericoides* (twig bark), *L.hookeri* (fruit), *L.virgatus* (bracts and sepals), *Lissanthe montana* (fruit), *Monotoca linifolia* (fruit), *Pentachondra pumila* (fruit), *Styphelia tubiflora* (flowers), *Trochocarpa cunninghamii* (fruit), *T.disticha* (fruit), *T.gunnii* (fruit; twig bark) and *T.thymifolia* (flowers; fruit).

Pigments from one native species from the Ericaceae i.e. *Pernettya tasmanica* (fruit) were also hydrolysed. The hexose present was galactose.

2. Identification of "Flavonoids"

Thirty five flavonols, or suspected flavonols, were detected in significant quantities in the family. Substitution occurred at position 3 excepting in two pigments in which it occurred at position 5. Evidence of 7 (or 4') substitution occurred mostly in minor constituents which were not considered in this present study. The majority of pigments were monosides but from chromatographic behaviour, there appeared to be several biosides.

Although structures were not determined for all pigments, positive or tentative identifications were ascribed to nineteen pigments. These included the 3-galactosides, 3-arabinosides, 3-glucuronides and 3-rhamnosides of kaempferol, quercetin and myricetin, the 3-rhamnosyl-glucosides of kaempferol, quercetin, myricetin and isorhamnetin (?), the 5-glucosides of kaempferol and quercetin, and quercetin 3-xylosyl-rhamnoside. The remaining 16 pigments have not been identified but spectral and hydrolytic information is available for 7 of these.

In addition to the flavonols detected, two dihydroflavones (flavanones), one chalcone and three dark coloured pigments (in UV light) of unknown identity were also observed. These pigments all appeared to have some taxonomic importance, and will be mentioned briefly below.

A representative chromatogram, showing the relative positions of the various pigments in BAW and 15% AcOH, is given in Fig. 8 (p. 49). The colour reactions of these pigments is given in Table 9 (p. 48).

Comments on Pigment Identification and Purification

In many cases, the presence of colourless contaminants including leucoanthocyanidins and catechins, complicated the purification of compounds. Successive or prolonged development on paper chromatograms did not always overcome this problem and resulted in a critical loss of material with some of the more labile pigments such as D_{26a} and D_{26b}.

Although not always apparent from chromatographic procedures, the presence of contaminants could be detected from spectral analyses, when the magnitude of spectral shifts and the position of spectral maxima were not in accordance with published data. This occurred with kaempferol ($AlCl_3$ and $AlCl_3$/HCl), myricetin (MeOH - band II), Qu-3-RhaGlc ($AlCl_3$), Qu-3-Gal from *Rhododendron* leaves ($NaOAc/H_3BO_3$), etc.

spot no.	pigment	UV	UV+NH_3
1	D_{26a}	-	Y
2	D_{26b}	-	YGr
3	D_{37}	dk	Y
4	D_{47}	dk	Y
5	Qu-5-Glc	flY	flY
6	Km-5-Glc	flY	flY
7	My-3-Glur	dk	YO
8	D_{34}	dk	Y
9	My-3-RhaGlc	dk	YO
10	D_{42}	dk	Y
11	D_{43}	dk	Y
12	D_{44}	dk	Y
13	D_{30}	dk	YGr
14	My-3-Gal	dk	YO
15	My-3-Arab	dk	YO
16	D_{20}	dk	YO
17	Ch_1	dk	O
18	Qu-3-Arab	dk	Y
19	Qu-3-Gal	dk	Y
20	D_{50}	dk	YO
21	Qu-3-Glur	dk	Y
22	Qu-3-RhaGlc	dk	Y
23	D_{38}	dk	Y
24	D_{39}	dk	Y
25	Qu-3-XylRha	dk	YBr
26	Km-3-RhaGlc	dk	YGr
27	My-3-Rha	dk	YO
28	Km-3-Glur	dk	YGr
29	Qu-3-Rha	dk	YBr
30	Km-3-Gal	dk	pY
31	D_{31}	dk	Y
32	Km-3-Arab	dk	YGr
33	Pc/Black	dk	dk
34	D_{18}	dk	Y
35	D_{32}	dk	YGr
36	Km-3-Rha	dk	dYGr
37	Br_1	dk	dk
38	Br_2	dk	dk
39	OPi_1	OPi	pY
40	OPi_2	OPi	pY

Table 9. "Flavonoids" detected in the Epacridaceae (see Fig. 8).

(dk = dark absorbing, fl = fluorescent, p = pale, Br = brown, Gr = green, O = orange, Pi = pink, Y = yellow)

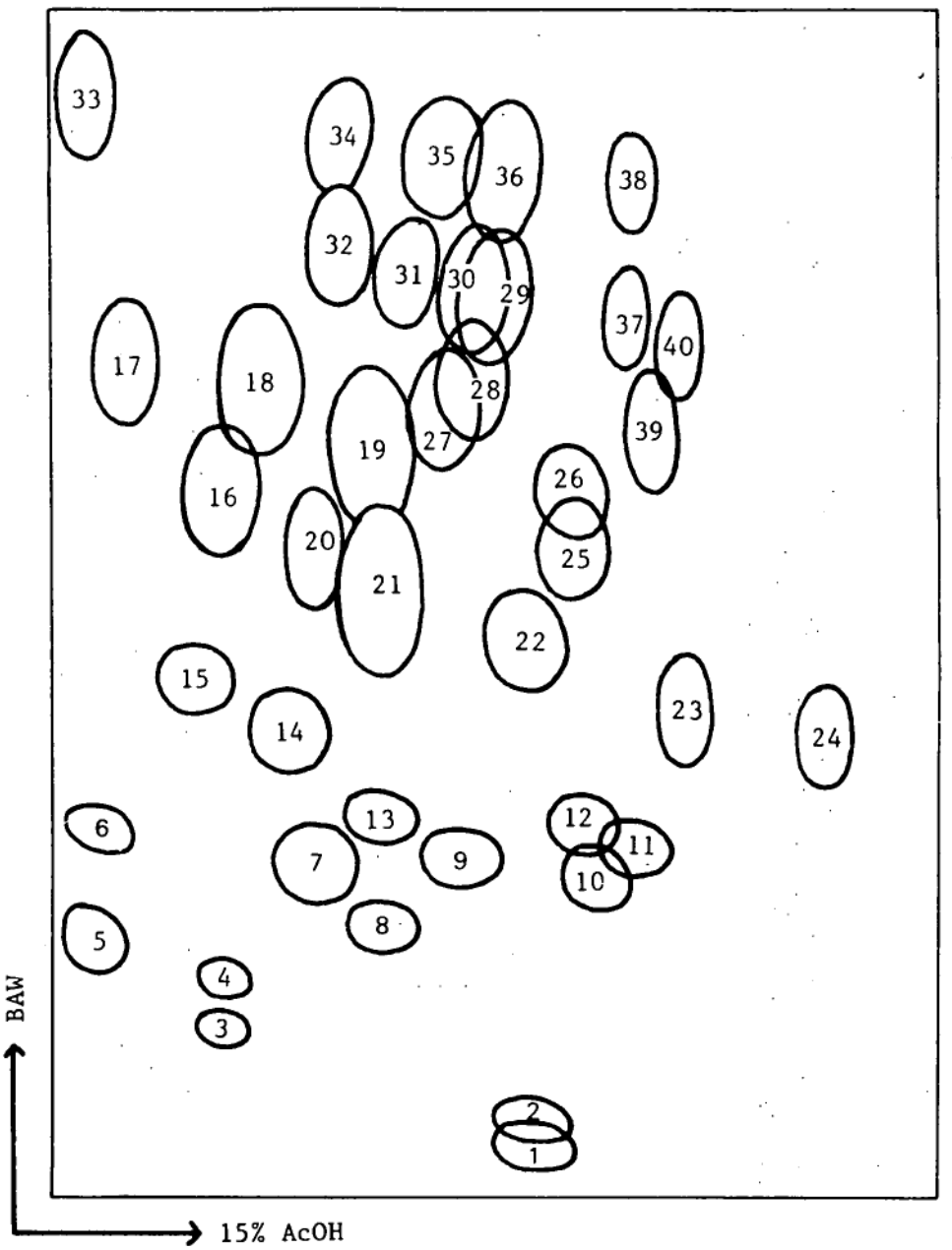


Fig. 8. Chromatographic position of Epacrid "flavonoids". (The legend is given as Table 9, facing page.)

Unfortunately, when the identity of a compound is not known or is not suspected, the distinction between shifts due to structural characteristics and those due to contaminants cannot be determined readily unless the shape of the spectra is completely aberrant. Hence, there is a need for an efficient purification technique comparable with that available for anthocyanins (see p. A7 - A8).

The MeOH spectra of 19 pigments isolated from the Epacridaceae is given in Appendix A (pp. A38 - A45). The reaction to specified reagents is also recorded. Species from which pigments were positively identified by hydrolytic and spectral procedures are shown in Table 10 (p. 51).

Throughout the analysis of flavonoid compounds, it was observed that myricetin derivatives were more susceptible to breakdown than their quercetin and kaempferol analogues.

(a) Leucoanthocyanidins

The presence of leucoanthocyanidins was deduced from the detection of anthocyanidins in acid hydrolysed extracts of non anthocyanin coloured tissues.

Only 2 leucoanthocyanidins, leucocyanidin and leucodelphinidin, were observed. The occurrence of these pigments had been investigated previously in 24 species, during research submitted for an Honours degree. The work has been repeated in this study.

(b) Flavonol aglycones

The identification of 3 aglycones, kaempferol, quercetin and myricetin, was also determined during previous research (for an Honours degree) but was confirmed in this research. Sufficient myricetin was not available to test its reaction with the reagents NaOAc and H_3BO_3 in the spectral analyses, but the appropriate information was available from the spectra of the glycoside (My-3-galactoside).

(c) Flavonol 3-glucuronides

The identity of glucuronic acid, as distinct from galacturonic acid, was not proven unequivocally in any compound detected in the survey. In the chromatographic analysis of sugars, both have a very low mobility in BBPW and do not separate. In this work, pigments containing sugar molecules of the appropriate R_f have been referred to as glucuronides rather than galacturonides because glucuronic acid has been reported more frequently in flavonoids than galacturonic acid and it has been reported (although rarely) in the related family, the Ericaceae (Harborne - 1967). In view of the widespread occurrence of

Compound	Species	Tissue
Qu-3-galactoside	*Epacris impressa*	leaves
	Richea pandanifolia	leaves
	R.dracophyllum	leaves
	R.procera	anthers
	Cyathodes juniperina	leaves
	C.petiolaris	fruit
	Monotoca submutica	leaves
Qu-3-arabinoside	*Epacris impressa*	leaves
	Richea procera	anthers
	Monotoca submutica	leaves
Qu-3-glucuronide	*Prionotes cerinthoides*	flowers
	Cyathodes juniperina	leaves
	C. var.*pendulosa*	flowers
	Leucopogon ericoides	leaves
Qu-3-rhamnoside	*Epacris tasmanica*	flowers
	Cyathodes juniperina	leaves
Km-3-galactoside	*Cyathodes juniperina*	leaves
Km-3-arabinoside	*Richea procera*	anthers
Km-3-glucuronide	*Leucopogon ericoides*	leaves
Km-3-rhamnoside	*Epacris tasmanica*	flowers
My-3-galactoside	*Epacris lanuginosa*	leaves
My-3-glucuronide	*Leucopogon ericoides*	leaves
Qu-3-rhamnosylglucoside	*Cyathodes juniperina*	leaves
Is?-3-rhamnosylglucoside	*Cyathodes juniperina*	leaves
Qu-3-xylosylrhamnoside	*Prionotes cerinthoides*	flowers
Qu-5-glucoside	*Richea procera*	anthers

Table 10. Flavonols positively identified in the Epacridaceae, and their source.

galactose in the family, this deduction may subsequently prove to be incorrect. However, providing both sugars are not present in the family, the taxonomic implications of glucuronide distribution will remain unchanged even though the structure may be different from that believed at present.

(d) Flavonol 3-arabinosides

The identity of the sugar moiety in Qu-3-Arab was determined using standard hydrolytic procedures. However, the chromatographic behaviour of the parent compound was not consistent with that given by Harborne (1967) for Qu-3-Arab (avicularin) and the pigment does not

co-chromatograph favourably with Qu-3-Arab obtained from *Rhododendron* leaves. (The species of *Rhododendron* is uncertain but it is likely to be a cultivar of *R.arboreum*. In view of the widespread occurrence of avicularin in *Rhododendron* species - Harborne and Williams (1971) - it seems reasonable to assume its identity in the unnamed species used here, particularly as its relative chromatographic behaviour fits that given by Harborne - 1967.) These results suggest that either the ring form of the sugar, or the aglycone-sugar link is different in the Epacrid pigment. It seems that the former alternative is more probable, since the chromatographic behaviour of this pigment is consistent with that reported for foeniculin (Harborne and Saleh - 1971) from *Foeniculum vulgare* (Umbelliferae). It is interesting to note that in *F.vulgare*, Qu-3-Arab is also accompanied by flavonol glucuronides.

Analagous kaempferol and myricetin 3-arabinosides are also present in the Epacridaceae. In the case of My-3-Arab, the identity of the sugar moiety was not confirmed but its chromatographic behaviour was consistent with its suggested identity.

Whilst the common form of Qu-3-Arab in the family is probably foeniculum, it seems probable that avicularin also occurs, but rarely in significant quantities. In *Monotoca scoparia*, a yellow pigment (in UV with NH_3) of appropriate R_f was present on 2-D chromatograms but was confused by the occurrence of a yellow-green kaempferol glycoside of similar R_f (Km-3-Arab in which the structure of arabinose was the same as in foeniculum). Corresponding kaempferol and myricetin derivatives (D_{18} and D_{20} respectively) showing the analagous chromatographic behaviour were also present in the family.

(e) Qu-3-rhamnoside and Km-3-galactoside

The identity of Qu-3-Rha was determined unambiguously by spectral, chromatographic and hydrolytic procedures. Confirmation of its identity was obtained by co-chromatography with quercetrin (commercial).

Km-3-Gal was obtained as a contaminant in Qu-3-Rha isolated from *Cyathodes juniperina* (leaves). Hydrolysis of the contaminated pigment yielded quercetin, kaempferol, rhamnose and galactose. Since the chromatographic behaviour of Qu- and Km-3-Rha was already known, the identity of the contaminant was deduced as Km-3-Gal. Published R_f data supported this identification.

The two pigments, Qu-3-Rha and Km-3-Gal, have similar R_fs and do not separate on 2-D chromatograms. Their colour reactions are different but when present as minor constituents, they are not differentiated

easily. Even as major constituents, the presence of one may obscure the presence of the other. This obviously occurred in several species, and there is a conspicuous absence of Km-3-Gal throughout the survey. Furthermore, kaempferol was detected on the aglycone papers for 7 species in which kaempferol derivatives were not recorded on the corresponding 2-D glycoside chromatograms. (These species were *Epacris longiflora* - flowers, *E.microphylla*, *Richea milliganii*, *Brachyloma ciliata*, *B.daphnoides*, *B.depressum* and *Leucopogon amplexicaulis* - leaves.) In each case, a pigment was observed in the appropriate position for either Km-3-Gal or Qu-3-Rha but was recorded as the latter.

Species in which either or both of these pigments were recorded require a more detailed investigation.

(f) My-3-rhamnoside

The identity of My-3-Rha was deduced on the basis of its chromatographic behaviour and co-occurrence with Km- and Qu-3-Rha. This identification has yet to be confirmed by hydrolytic procedures.

(g) Qu-3-xylosylrhamnoside

Qu-3-XylRha has not been previously reported and represents the first record of this disaccharide as a substituent in flavonols. The identification is as yet incomplete, but spectral data indicates that substitution occurs at position 3 and not at positions 5 or 7. Hence, the sugar moiety is almost certainly a disaccharide. The order of sugar attachment to the aglycone is not known but the widespread occurrence of rhamnosides would suggest that rhamnose is attached directly to quercetin.

(h) Km-, Qu-, My- and Is(?)-3-RhaGlc

Qu- and Is(?)-3-RhaGlc were inseparable using descending chromatography but because of their low R_f in BAW, circular chromatography was used successfully. Confirmation of the identity of Qu-3-RhaGlc was obtained by co-chromatography with rutin (commercial).

The structure of Km- and My-3-RhaGlc was not determined but their colour and chromatographic behaviour (in comparison with published data) was acceptable for their proposed structure.

The aglycone present in Is(?)-3-RhaGlc has not been determined unambiguously. Spectral data from the glycoside suggests that a free 5, 7 and 4' hydroxy group is present but chromatographic behaviour clearly shows that the aglycone is not kaempferol. Its R_f in Forestal is consistent with that of rhamnetin but the colour is different. From published data (Harborne - 1967), both isorhamnetin (3'-OMe of quercetin)

and syringetin (3',5'-diOMe of quercetin) have a similar R_f to rhamnetin.

Isorhamnetin is the most common methylated flavonol aglycone reported in nature, and the R_f of its glycoside would be expected by analogy to resemble that of quercetin more closely than would the R_f of syringetin. (Malvidin glycosides resemble cyanidin glycosides more closely than do petunidin glycosides). For these reasons, the aglycone in this pigment is tentatively identified as isorhamnetin.

Is-3-RhaGlc was isolated from *Cyathodes juniperina* leaves. It was not detected on 2-D chromatograms because of its similar R_f to Qu-3-RhaGlc. Hence, it is probably more widespread in the family than the survey would suggest.

(i) Qu- and Km-5-glucoside

The identity of Qu-5-Glc was determined rigorously, but the structure of Km-5-Glc was deduced on the basis of its colour reactions and co-occurrence with Qu-5-Glc.

The possibility of 5-substitution in these two compounds was suggested immediately by their intense yellow fluoresence in UV light. This was confirmed with Qu-5-Glc by standard spectral procedures and also by a comparison between the spectral shifts in $AlCl_3$/HCl (with respect to MeOH) of quercetin, quercetin 3-glycosides and quercetin 5-glucoside. According to Mabry *et al.* (1970), the bathochromic shift of 3,5-dihydroxyflavones (e.g. quercetin) is intermediate between that observed for their 3-hydroxy (e.g. Qu-5-Glc) and 5-hydroxy (e.g. Qu-3-glycosides) equivalents. The results obtained here are consistent with this notion.

(j) Pc/Black

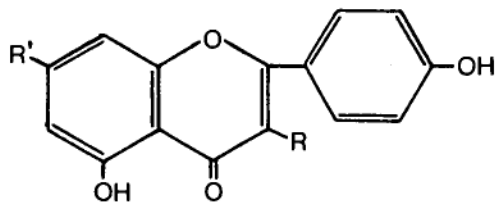
This compound was difficult to isolate in quantity because of its similar R_f to chlorophyll. However, relatively pure samples were obtained by successively running the chlorophyll band (from BAW) in H_2O. The leading edge of the pigment moved slightly in front of the chlorophyll band and could be accumulated from several runs.

Sufficient purified material was obtained to carry out spectral analyses, and the results are shown on p. A45 (Appendix A). The Band I maxima (in MeOH) suggests that the compound is either a flavone or a flavonol substituted at position 3. In view of the general absence of flavones in the family, the latter alternative would seem more likely. However, a positive Shinoda test for flavones (yellow colour with Mg ribbon) suggests otherwise.

On the addition of NaOMe, the position of Band I shifts

bathochromically but this shift is not accompanied by a decrease in intensity, indicating that a free 4'-OH (but not 3-OH) is present in the molecule. This is confirmed by the presence of a shoulder on the long wavelength side of Band I in NaOAc. The absence of a Band II shift with NaOAc indicates that a free hydroxyl group is not present at position 7. With $AlCl_3$, a bathochromic shift of both Band I and II is observed. This shift is stable on the addition of HCl indicating that position 5 is free and that there are no *ortho*-dihydroxy groups present. This latter point is also indicated by the absence of a Band I shift using H_3BO_3 in NaOAc.

Hence, from the information obtained here, the following structure may be derived -



where R = H or OMe or O-glycoside
and R' = H or OMe or O-glycoside.

Details at position 6, 8, 3' and 5' are not indicated from the spectra.

During the hydrolysis of this pigment, a precipitate formed as the reaction proceeded, indicating its insolubility in water (it was initially dissolved in MeOH). Analysis of the EtOAc extract showed 3 dark spots and 1 blue spot (in UV light) all with an R_f greater than kaempferol in Forestal and Formic Acid. No sugars were detected.

(k) OPi1 and OPi2

A positive Shinoda test (purple-pink) with OPi_1 indicated that this pigment was either a flavonol or a dihydroflavone. The shape and position of the spectral maxima in MeOH suggested the latter alternative and this was confirmed by its isomerization to a chalcone on the addition of NaOMe in the MeOH solution. This reaction also suggested that position 5 was substituted (Mabry *et al.* - 1970). However, an acid stable shift with $AlCl_3$ suggested the presence of a free 3 or 5 hydroxyl group and since dihydroflavonols do not appear to isomerize to chalcones, these results conflict with the possibility of 5-substitution. The absence of a Band II shift with NaOAc indicated that a free hydroxyl group was not present at position 7.

Characteristics of the B-ring are not indicated from the spectral

analysis because this ring is not conjugated with the A-ring in dihydroflavones.

Hydrolysis results indicated that glucose was involved in substitution but the position of attachment is unknown. A comparison of the aglycone and glycoside spectra (with respect to NaOAc) would seem to exclude position 7 from glycosylation.

The second pigment, OPi_2, is assumed to have a similar structure to OPi_1. It has a similar unusual colour reaction in UV light with NH_3 (orange pink → pale yellow), and similar chromatographic mobility and species distribution. However, hydrolysis of this pigment also yielded glucose, suggesting that the position of substitution must be different since the same sugar is involved.

(l) D34 and D30

Purification of these two pigments was complicated by the presence of leucoanthocyanidins, and attempts to identify them were unsuccessful. Hydrolysis of D_{30} yielded quercetin and cyanidin but no sugars were observed.

Their colour reactions in UV light with NH_3 (i.e. dark → yellow and yellow-green respectively) and their chromatographic behaviour suggest that they are monosides substituted at position 3.

(m) D26a and D26b

These two compounds have a particularly low R_f in BAW, accompanied by a relatively high R_f in 15% AcOH, suggesting that they are either biosides or triosides.

Attempts were made to purify both pigments but they appeared to be particularly labile and sufficient contaminant free material was never obtained to carry out spectral analyses. A contaminated sample of D_{26a} was hydrolysed, yielding three aglycones i.e. quercetin, isorhamnetin(?) and cyanidin along with three sugars, galactose, glucose and glucuronic acid. Apart from the presence of leucocyanidin, it is difficult to derive any conclusions from these results.

The colour reactions of the two pigments in UV light and NH_3 (dark → yellow and yellow-green) are similar to those of D_{30} and D_{34}, and all four co-occur in *Cyathodes juniperina*. It seems probable that the structures of D_{30} and D_{34} are the same as those of the intermediates formed from the breakdown of D_{26a} and D_{26b} respectively.

(n) D21, D31, D32, D37, D38, D39, D42, D43, D44, D47, D50, Ch1, Br1, Br2

The identity of these 14 pigments is unknown, and no spectral or hydrolytic data are available for them at this stage. D21, D31, D32,

D_{37}, D_{47} and D_{50} are probably monosides whilst D_{38}, D_{39}, D_{42}, D_{43} and D_{44} are probably flavonol biosides or triosides. The colour reactions of these pigments are shown in Table 9 (p. 48), and are consistent with flavonols substituted at position 3.

From its colour reations, Ch_1 is assumed to be a chalcone. The class of compound to which Br_1 and Br_2 belongs is not known but from R_f and colour, they may be dihydroflavones (or dihydroflavonols). Their position on the chromatograms is not unlike that given for dihydroquercetin 3-arabinoside by Harborne and Williams (1971b).

3. Glycosidic Relationship (G.R.)

The glycosidic relationship offers a significant contribution to taxonomic and phylogenetic studies in the Epacridaceae. The term is used to indicate the accumulation of one glycosidic type in excess of another and will be written in the form x > y where x and y are the abbreviated forms of the sugars (see p. 41) involved in flavonoid glycosylation.

In this work, only the major glycosidic types in each chemical class have been considered in the derivation of G.R. In anthocyanins, galactosides and arabinosides are characteristic. Thus, a G.R., Gal > Arab, indicates that anthocyanidin galactosides are accumulated in excess of anthocyanidin arabinosides. Where rhamnosylgalactosides are accumulated in excess of either galactosides or arabinosides, G.R. is given as Gal > Arab. This situation is only applicable to the Styphelieae fruits, and an examination of anthocyanins in other organs of these species suggests that this interpretation is correct.

In flavonols, the major glycosidic types are galactosides and glucuronides. Although arabinosides are common and are considered characteristic of the family, they are rarely accumulated in excess of galactosides (see p.78). Rhamnosides are also common, but their occurrence as dominant pigments is mostly restricted to the floral tissues. There appears to be no correlation between the accumulation of rhamnosides and other glycosides, and even where they are dominant, their presence has been ignored and G.R. is still estimated between galactosides and glucuronides.

Interpretation of the G.R. with flavonol biosides is more complicated than with anthocyanins because the hexose (glucose) contained in the bioside is different from that in the monosides (galactose and glucuronic acid). Logically, it would be expected that dominant rhamnosylglucosides

should be grouped with glucuronides but in fact they seem to be associated with either glucuronic acid or galactose e.g. see *Archeria hirtella*, *Acrotriche serrulata*, *A.divaricata* and *Trochocarpa gunnii* (Table A5, p. A22). However, the incorporation of glucose into the biosides may effect (i.e. reduce, in the case of *Archeria hirtella* and *Acrotriche divaricata*) the incorporation of glucuronic acid in the monoside. This would again suggest that rhamnosylglucosides should be grouped with glucuronic acid in the determination of G.R. Under these circumstances, *Archeria hirtella* would then be consistent in its G.R. with other species of the genus, and *Acrotriche divaricata* would be comparable with *A.serrulata*. Furthermore, this would establish a connection between inter-population variation of glucuronides and rhamnosylglucosides observed in *Archeria eriocarpa* and *Cyathodes juniperina*. In the former species, rhamnosylglucosides were dominant in samples from Adamsons Peak (south) whilst glucuronides were dominant in samples from Howard's Road (west). With *Cyathodes juniperina*, rhamnosylglucosides were generally dominant in highland areas (Mt. Rufus canal, Hartz Mts.) whilst glucuronides were dominant in the coastal population examined from Tasman Peninsula (south east). Also with this species, parallel variation between anthocyanidin galactosides and rhamnosylgalactosides was observed. This variation may have been due to a variable degree of breakdown of the bioside, but more probably it is related to the response to different habitat conditions.

4. Distribution of Flavonoids within the Epacridaceae

One hundred and thirteen species from the Epacridaceae were examined for anthocyanins and/or 'flavonoids'. The general distribution of these pigments is given in Table A4 (anthocyanins) and Table A5 ('flavonoids') on pp. A16 and A22 respectively of Appendix A. In order to standardize the comparison between species, only those compounds detected on 2-D chromatograms are scored. (In Table A4, minor constituents identified during isolation procedures but not observed on 2-D chromatograms are placed in the final column of the table.) Chemical patterns given for many species, particularly those of Table A5, have been compiled from several chromatograms, each representative of a different location i.e. individual intra-specific variation has been ignored in these composite chromatogram patterns. This has undoubtedly led to some errors in the estimation of the quantitative relationships between the pigments but it has led to no alteration of the characteristic G.R.

A summary of Tables A4 and A5 with respect to the more widespread pigments is given in Tables 11 - 14, where the frequency of these compounds is shown. Figures given in the tables represent minimum frequencies since all species were not considered in the same detail i.e. some were scanned by 2-D chromatography only, whilst others were involved in full scale isolation procedures. Furthermore, samples from several populations were available for some species but not for others (see Tables A4 and A5).

Eighty five species were examined for anthocyanins, and cyanidin was detected in all (100%), delphinidin in 39 species (46%), pelargonidin in 6 (7%) and malvidin in one species (1%) - see Table 11. These figures do not compare favourably with those quoted by Harborne (1967) for the Australian flora as a whole, in which the occurrence of delphinidin (63%) exceeds that of cyanidin (37%). The relative scarcity of delphinidin in the Epacridaceae is not surprising however, in view of the widespread occurrence of white or pink reproductive tissues and the scarcity of blue or purple tissues i.e. with the exception of a few species having purple fruit in the Styphelieae, the occurrence of delphinidin in the family is mainly due to the contribution of young twig bark pigments. Pelargonidin is rare in the family and this is consistent with its low occurrence in other families in Australia.

	frequency (%)				No.
Tissue	Cy	Dp	Pel	Mv	of spp.
Subfamily Epacrideae					
flowers	100	14	3	3	35
leaves	100	3	0	0	27
twig bark	100	53	0	0	15
capsules	100	0	0	0	6
all tissues included	100	31	2	2	42
Subfamily Styphelieae					
flowers	100	17	7	0	29
leaves	100	33	0	0	21
twig bark	100	72	0	0	18
fruit	100	60	13	0	25
all tissues included	100	55	12	0	43
Total Family					
flowers	100	16	5	2	64
leaves	100	31	0	0	48
twig bark	100	65	0	0	34
fruit	100	50	10	0	31
all tissues included	100	46	7	1	85

Table 11. Frequency of anthocyanidins in the Epacridaceae.

Harborne (1967) has suggested that its rarity in the Australian flora has resulted from the low incidence of bird pollination (and resultant lack of selection for highly coloured floral tissues). Malvidin is extremely rare in the Epacridaceae but its presence, rather than that of either peonidin or petunidin would agree with Harborne's remark that malvidin is more widespread than either of the alternative methylated anthocyanidins.

One hundred and thirteen species were examined for 'flavonoids', and the three common aglycones i.e. quercetin, kaempferol and myricetin were detected in 112 species (99%), 107 (95%) and 74 species (66%) respectively. The occurrence of these aglycones in leaves, flowers and fruit is given in Table 12, along with a comparison of dicots in general (taken from Harborne - 1967). The general trend i.e. quercetin > kaempferol > myricetin, is apparent throughout excepting in the fruit tissue where the occurrence of myricetin is considerably higher than that of kaempferol.

Tissue	frequency (%) Qu	Km	My	No. of spp.
Subfamily Epacrideae				
leaves	98	83	49	53
flowers	100	97	33	39
all tissues included	98	94	55	53
Subfamily Styphelieae				
leaves	100	92	62	60
flowers	96	87	41	46
fruit	88	46	82	33
all tissues included	100	95	75	60
Total Family				
leaves	99	88	56	113
flowers	98	92	38	85
both tissues included	99	95	66	113
Dicotyledons				
leaves	62	52	11	
flowers (cyanic)	68	48		

Table 12. Frequency of flavonol aglycones in the Epacridaceae and a comparison with Dicots in general (taken from Harborne - 1967).

The frequency of anthocyanins and flavonol glycosides is shown in Tables 13 and 14 (p. 61) respectively. These results are diagrammatically represented by histograms in Figs. 9 and 10 (pp.62 - 64) respectively. In Table 14 and Fig. 10, those pigments occurring in Table A5 with a frequency of less than 10% in all organs examined, have

been omitted.

An examination of these tables and histograms indicates that the characteristic glycosides of the family are galactosides and arabinosides in anthocyanins, and galactosides, arabinosides and glucuronides in flavonols. Rhamnosides are also common in flavonols but as dominant pigments, they are mostly restricted to floral tissues.

Tissue	Cy3Gal	Cy3Arab	Cy3Glc	Cy3Rha	Cy3RhaGal	Cy3RhaGlc	Cy3Xy1Arab	Cy3Xy1Gal	Dp3Gal	Dp3Arab	Dp3Glc	Dp3RhaGal	Dp3RhaGlc
Subfamily Epacrideae													
leaves (27 spp.)	100	100							22	30			
flowers (35)	100	100		3	9			3	14	17			
capsules (6)	100	100						17					
twig bark (15)	100	100							47	53			
Subfamily Styphelieae													
leaves (21 spp.)	90	86	10		24	14			33	10		5	
flowers (29)	93	86	7		28	7	3		10	3			
fruit (25)	88	88	8	16	56	8	4	4	36	16	8	24	8
twig bark (18)	94	89	6		28	6			50	33	6	6	6
Total Family													
leaves (48 spp.)	96	94	4		10	6			27	21		2	
flowers (64)	97	94	3	2	17	3	2	2	13	11			
fruit (31)	90	90	6	13	45	6	3	6	29	13	6	19	6
twig bark (34)	97	94	3		15	3			50	44	3	3	3

Table 13. Frequency of major anthocyanins in the Epacridaceae.

Tissue	Qu3Gal	Qu3Glur	Qu3Arab	Qu3Rha	Km3Gal	Km3Glur	Km3Arab	Km3Rha	My3Gal	My3Glur	My3Arab	My3Rha	Qu3RhaGlc	Km3RhaGlc	My3RhaGlc	D30	D34	D50
Subfamily Epacrideae																		
leaves (53 spp.)	98	62	94	62	17	42	34	32	45	40	28		9	4	8	2	9	6
flowers (39)	82	62	59	90	0	10	41	87	23	26	5	23	10	10	0	0	8	0
Subfamily Styphelieae																		
leaves (60 spp.)	85	62	67	77	7	53	12	68	55	47	30	7	27	15	12	10	8	8
flowers (46)	67	61	35	52	11	22	9	80	15	30	4	4	37	9	15	11	11	
fruit (33)	52	64	36	61		18	9	21	33	61	21	12	24	12	24	9	9	3
Total Family																		
leaves (113 spp.)	91	62	80	70	12	48	22	51	50	43	29	4	19	10	10	6	9	7
flowers (85)	74	61	46	69	6	16	24	84	19	28	5	13	25	9	8	6	9	

Table 14. Frequency of major flavonols in the Epacridaceae.

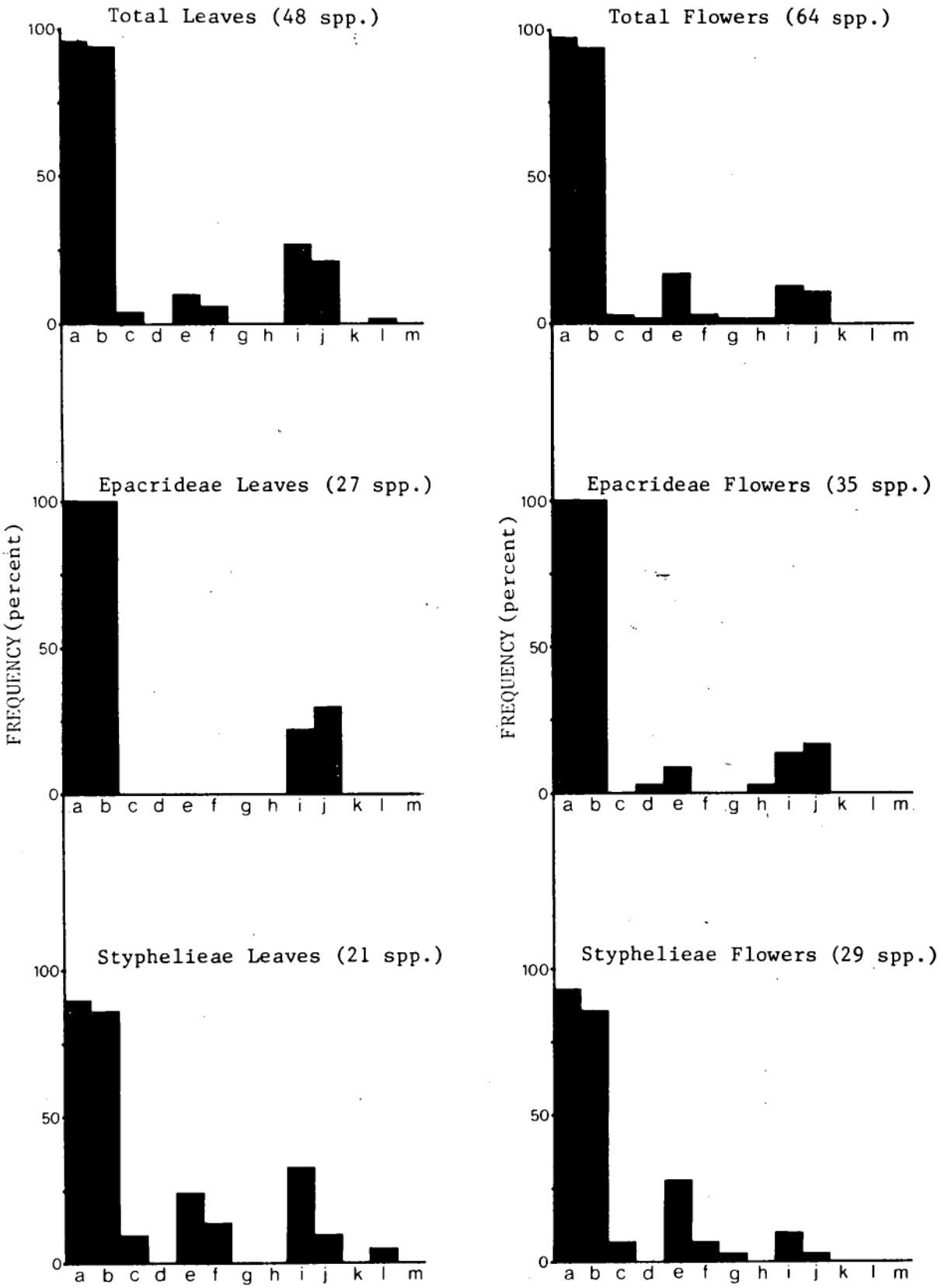
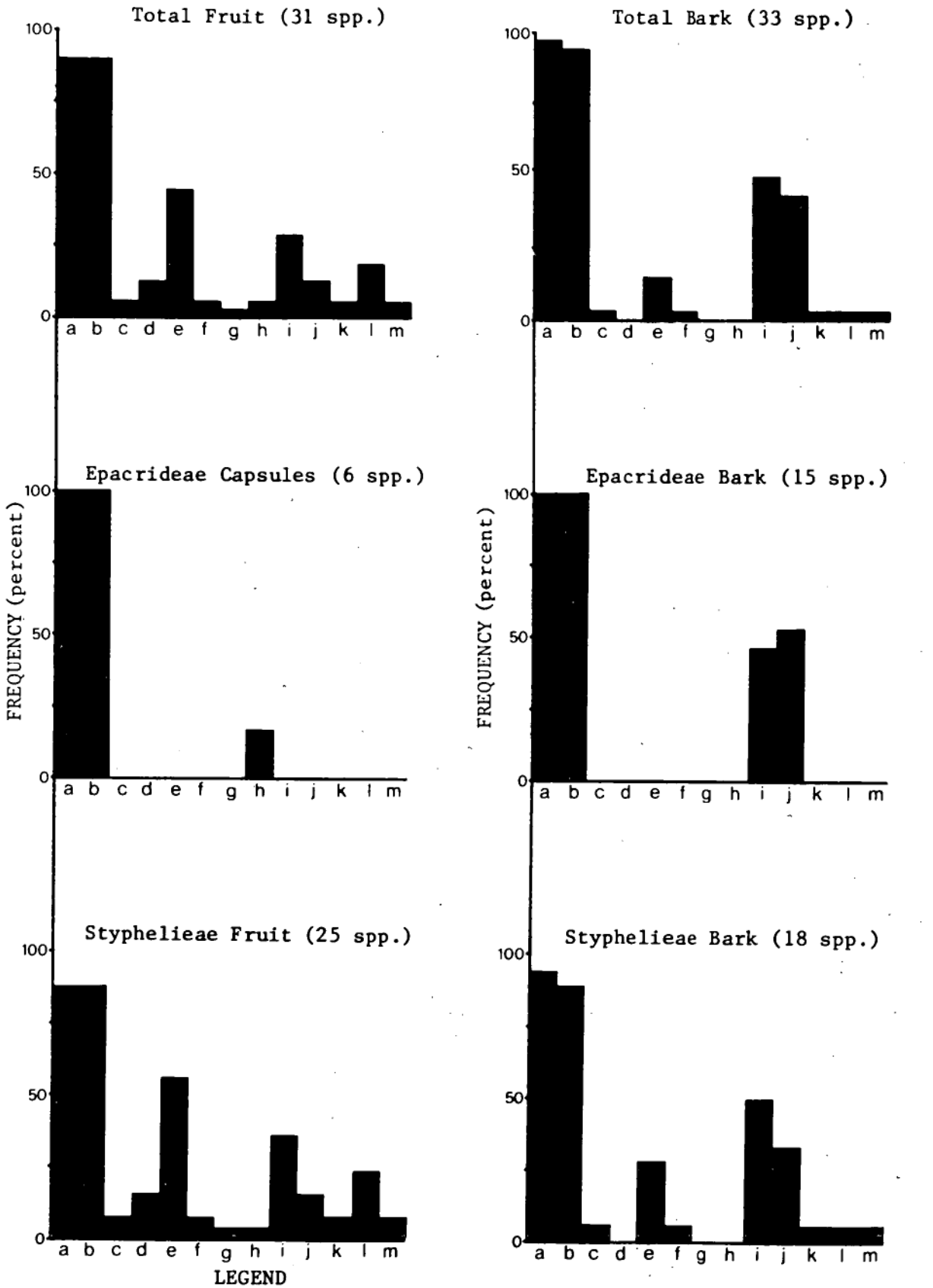


Fig. 9. Frequency of major anthocyanins in the Epacridaceae.

75-3-95


Total Fruit (31 spp.)
Total Bark (33 spp.)
Epacrideae Capsules (6 spp.)
Epacrideae Bark (15 spp.)
Styphelieae Fruit (25 spp.)
Styphelieae Bark (18 spp.)
FREQUENCY (percent)
100
50
0
a b c d e f g h i j k l m
LEGEND
a = Cy-3-Gal
b = Cy-3-Arab
c = Cy-3-Glc
d = Cy-3-Rha
e = Cy-3-RhaGal
f = Cy-3-RhaGlc
g = Cy-3-XylArab
h = Cy-3-XylGal
i = Dp-3-Gal
j = Dp-3-Arab
k = Dp-3-Glc
l = Dp-3-RhaGal
m = Dp-3-RhaGlc

25-3-96

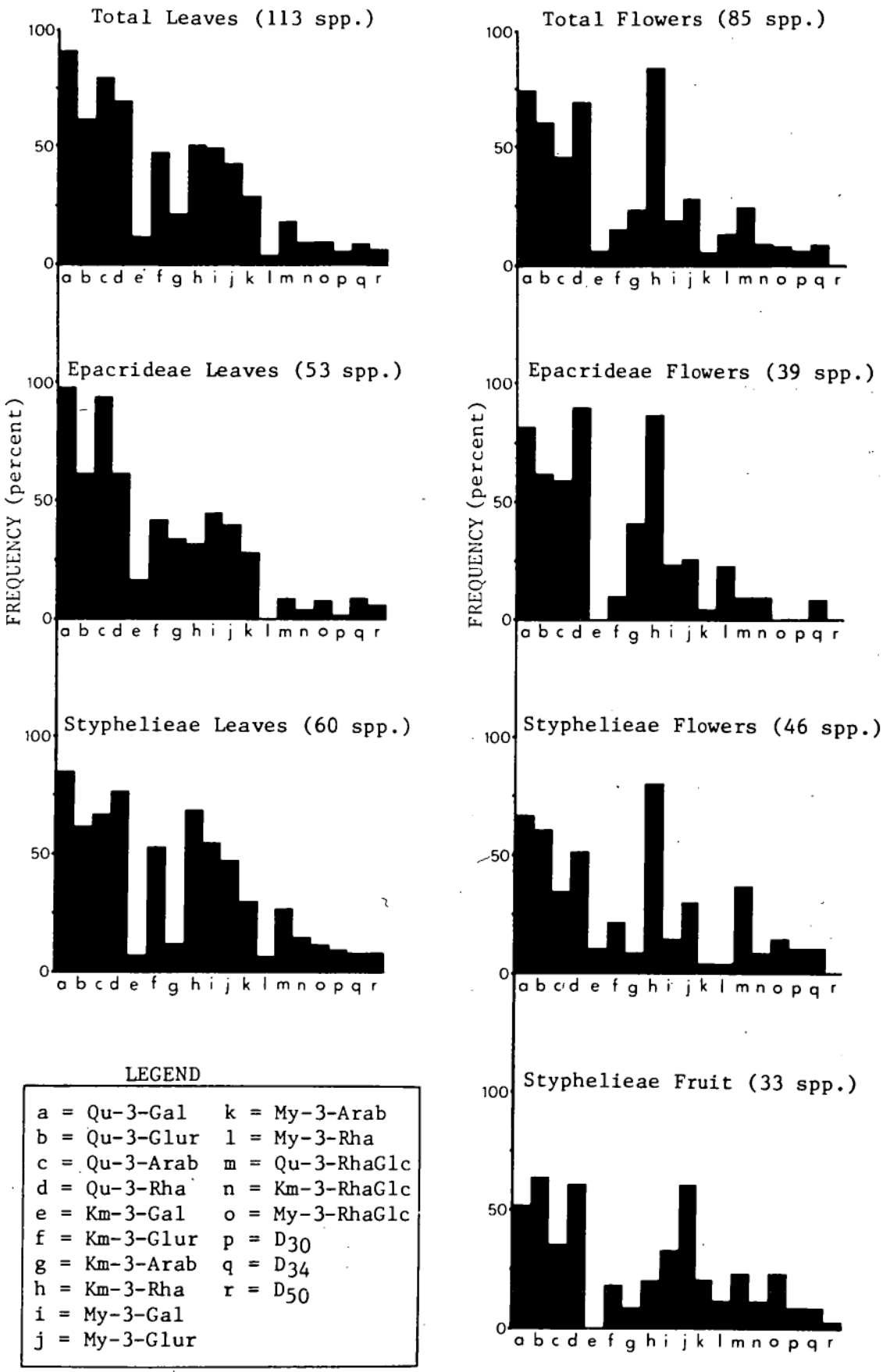


Fig. 10. Frequency of major flavonols in the Epacridaceae.

75-3-94

The occurrence of leucoanthocyanidins was investigated in the leaves of 68 species. Leucocyanidin was found in all, and leucodelphinidin in 74% (50 spp.). Although the flowers from 40 species were also tested for these compounds, the situation was complicated by the presence of anthocyanins which, on hydrolysis, yield the same aglycones as the analagous leucoanthocyanidins. Cyanidin was detected in all flowers examined but the origin of this aglycone cannot be determined with certainty using the procedures adopted in this work. However, because of the relative scarcity of delphinidin derivatives in the flowers, the presence of leucodelphinidin can be ascertained with more confidence. Consequently, after excluding the only species found to accumulate delphinidin glycosides, 14 (36%) of the 39 remaining species yielded delphinidin among the hydrolysis products.

The distribution of leucocyanidin and leucodelphinidin is shown in Table A6 (p. A34). A summary of this table is given below.

Tissue	frequency (%) leucocyanidin	leucodephinidin	No. of spp.
Subfamily Epacrideae			
leaves	100	73	33
flowers	100 (?)	47	17
Subfamily Styphelieae			
leaves	100	74	35
flowers	100 (?)	27	22
Total Family			
leaves	100	74	68
flowers	100 (?)	36	39

Table 15. Frequency of leucoanthocyanidins in the Epacridaceae.

(a) Subfamily distribution

Species of the subfamily Styphelieae show greater anthocyanin diversity than species of the subfamily Epacrideae. This is apparent, not only in the number and structure of pigments but also in the anthocyanin patterns for individual species. Cyanidin derivatives occur in all species examined from both subfamilies but delphinidin and pelargonidin derivatives occur in only 31% (13 spp.) and 2% (1 sp.) respectively compared with 55% (23 spp.) and 12% (5 spp.) respectively in the Styphelieae.

Nine anthocyanins were present in the Epacrideae (see Table A4, p. A16)

but only 4 (all monosides) were present in significant quantities and 2 of these (the delphinidin derivatives) were major pigments in the bark only. The five minor constituents included 2 cyanidin biosides, one pelargonidin and 2 malvidin monosides. The pelargonidin and malvidin glycosides were each found in only one species. Delphinidin and pelargonidin biosides were not detected in the Epacrideae. In the Styphelieae, 18 anthocyanins were detected and 10 were present in significant quantities, including biosides and delphinidin derivatives. Although pelargonidin derivatives were minor compounds, they were more widespread (5 spp.) and showed greater structural variation than was apparent in the Epacrideae.

Clear cut differences between the two subfamilies are less pronounced with flavonols but the results nevertheless support those observed for the anthocyanins. Twenty nine flavonols (8 biosides) and 2 dihydroflavones were detected in the Epacrideae. However, 8 of these including 4 biosides, were species-specific and 4 others, including one bioside, were not observed in significant quantities. (For the purposes of this comparison, "significant quantities" implies a concentration greater than '+' in Table A5.) In the Styphelieae, 27 flavonols (6 biosides), one chalcone and 2 probable dihydroflavonols were detected. Again however, 5 compounds including 3 biosides and one chalcone, were species-specific and another pigment was not observed in significant quantities in any species. With the exclusion of very minor constituents of infrequent occurrence, and species-specific compounds, neither of which may be regarded as characteristic, pigments detected in significant quantities in the two subfamilies include 17 flavonols (3 biosides) and 2 dihydroflavones in the Epacrideae and 22 flavonols (3 biosides) and 2 probable dihydroflavonols in the Styphelieae.

The 3 common aglycones, kaempferol, quercetin and myricetin, are represented in both subfamilies but the occurrence of myricetin is more restricted in the Epacrideae than in the Styphelieae (see Table 12, p. 60).

(b) Generic distribution

Of the 5 common flavonoid aglycones, cyanidin and quercetin are present in all genera studied, and with the exception of *Lebetanthus*, the same is true for kaempferol. Myricetin is widespread but is absent from three genera, *Archeria*, *Lebetanthus* and *Brachyloma*. The occurrence of delphinidin is less common than the other 4 aglycones and in most species, its recorded presence in leaves and flowers is due to

contamination from traces of twig bark. Apart from the twig bark, delphinidin derivatives are common only in *Trochocarpa* and *Cyathodes*.

The number of glycosides detected in each genus varies, with 7 in *Brachyloma* being the smallest (5 flavonols, 2 anthocyanins) and 35 in *Cyathodes* being the highest (22 flavonols, 12 anthocyanins and 2 probable dihydroflavonols). The variation in generic patterns with respect to 'flavonoid' glycosides is shown in Fig. 11 (p. 69). Anthocyanins have not been treated in this manner because of the relative uniformity of patterns (excepting in the Styphelieae fruit).

Cy-3-Gal and Cy-3-Arab are the only glycosides represented in every genus. Qu-3-Gal and Qu-3-Arab are present in all excepting *Styphelia* and Qu-3-Rha is present in all but *Prionotes*.

The distribution of anthocyanins and 'flavonoids' in each genus is shown in Table 16 (below) and 17 (p. 68), adapted from Tables A4 and A5.

Genus	No. of Spp.	Cy3Gal	Cy3Arab	Cy3Glc	Cy3Rha	Cy3RhaGal	Cy3RhaGlc	Cy3XylArab	Cy3XylGal	Dp3Gal	Dp3Arab	Dp3Glc	Dp3RhaGal	Dp3RhaGlc
Subfamily Epacrideae														
Archeria	4	4	4			1				1	1			
Dracophyllum	2	2	2											
Epacris	24	24	24			1				9	10			
Prionotes	1	1	1											
Richea	9	9	9			1			1					
Sprengelia	1	1	1											
Woollsia	1	1	1		1					1	1			
Subfamily Styphelieae														
Acrotriche	2	2	2			1				1	1			
Astroloma	1	1	1							1				
Brachyloma	1	1	1											
Cyathodes	11	11	11		4	7		1	1	5	3		2	
Leucopogon	11	11	11			3		1		3	2			
Lissanthe	3	3												
Monotoca	4	4	4			2				3	2		2	
Pentachondra	3	3	3			2				2	2			
Styphelia	2	2	2											
Trochocarpa	4	2	2	2		2	2			2		2	2	2

Table 16. Generic distribution of the major anthocyanins in the Epacridaceae.

Excluding the compounds mentioned above, the number of pigments shared by all members of any genus is very small. The possibility that this is due to the incomplete nature of the data can be ruled

Genus	No. of Spp.	Qu3Gal	Qu3Glur	Qu3Arab	Qu3Rha	Km3Gal	Km3Glur	Km3Arab	Km3Rha	My3Gal	My3Glur	My3Arab	My3Rha	Qu3RhaGlc	Km3RhaGlc	My3RhaGlc	Qu3Xyl1Rha	D30	D34	D50	D38	D18	D39	D31	D32	D20	D21	D26a, D26b	D37	OPi1, OPi2	D42,43,44	Pc/Black	D47	Br1, Br2
Subfamily Epacrideae																																		
Archeria	5	5	3	5	4		2	4	5					3	4																			
Dracophyllum	5	4	4	4	4		1	2	2	2	1	2							1		1													
Epacris	29	29	23	28	27	7	21	4	24	16	14	9	6	3		3		1	4	1									3	3	1		1	
Lebetanthus	1	1		1	1														1															
Prionotes	1	1	1	1			1	1		1	1			1		1	1															1		
Richea	10	10	5	10	10	1		10	6	6	4	3	1		2					3	3	2												
Sprengelia	1	1	1	1	1		1	1	1		1		1																					
Woollsia	1	1		1	1			1	1	1	1	1	1																					
Subfamily Styphelieae																																		
Acrotriche	2	2	1	2	2		1		2	2	2	2		2	2	2																		
Astroloma	2	2	1	1	2		1		1		1						1				1													
Brachyloma	3	3		3	3				2														1											
Cyathodes	12	12	10	10	12		8	2	10	1	10	9	6	7	3	4	2	4	4	1				1				1	1					1
Leucopogon	17	14	12	12	16	3	10	3	15	8	7	5	1	4	2		1			1	1			2		1								
Lissanthe	3	3		3	2	3	1	2	3	1		1			1																			
Monotoca	9	9	6	4	6	1	5	3	9	9	5	3	2	3		4		3	3	4	1	1		3	5	1								
Pentachondra	3	3	2	3	2		1		3	2	2			2	1																			
Styphelia	4		4		3		4				2			2		2																			
Trochocarpa	5	5	3	5	5	1	2		4	3	4	3	1	4	2	4								1		1								

Table 17. Generic distribution of 'flavonoids' in the Epacridaceae.

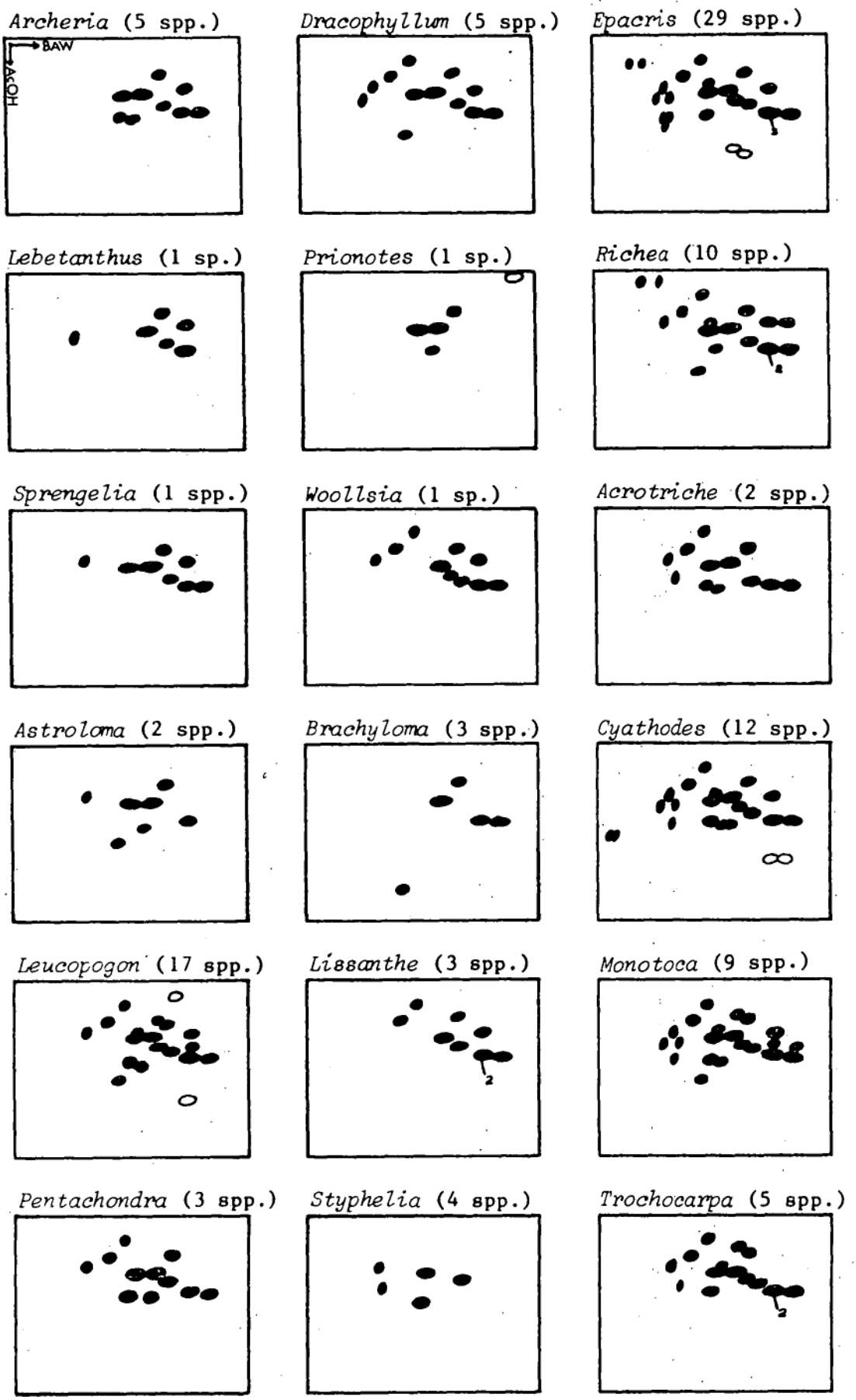


Fig. 11. "Flavonoid" patterns in genera of the Epacridaceae.

out from an examination of Table 18 which shows the distribution of 'flavonoids' in comparable tissues. (Monotypic genera and those in which only one species were considered, have been excluded.) In only 4 genera (*Styphelia* - leaves and flowers, *Lissanthe* - flowers, *Brachyloma* - leaves, *Archeria* - flowers) do all members share at least half of the compounds known to occur in the genus for the particular tissue under consideration.

Genus	flowers		leaves		fruit	
	sp.n.	sh.c	sp.n.	sh.c	sp.n.	sh.c
Subfamily Epacrideae						
Archeria	4	6/9	5	1/8		
Dracophyllum			5	0/12		
Epacris	23	2/12	29	1/23		
Richea	8	2/14	10	3/12		
Subfamily Styphelieae						
Acrotriche	2	1/7	2	7/12		
Astroloma	2	0/1	2	0/8	2	0/2
Brachyloma			3	3/5		
Cyathodes	11	0/18	12	1/18	11	0/21
Leucopogon	12	1/13	17	0/20	6	0/11
Lissanthe	3	4/6	3	3/9	3	2/7
Monotoca			9	2/21	4	0/12
Pentachondra	3	1/9	3	2/10		
Styphelia	2	5/5	4	2/4		
Trochocarpa	3	4/10	5	3/15	4	0/4

Table 18. The number of 'flavonoids' shared by all members of each genus in comparable tissues. (sp.n. = number of species examined from the genus, sh.c = shared compounds i.e. x/y where x = the number of compounds shared by all species and y = the total number of compounds observed.)

(c) Tissue distribution

Anthocyanins

The degree of anthocyanin pigmentation varied extensively between species and is related to seasonal and physiological stages in the plant, as well as to tissue differences.

Many species are characterized by a seasonal "flush" in which the young, rapidly developing tissues are highly pigmented during their main growing period. This was generally more widespread in twig bark but a few species e.g. *Trochocarpa gunnii, T.cunninghamii, Prionotes cerinthoides* also produce strong pigmentation in the new young leaves. Anthocyanins fade within a few weeks leaving the tissues acyanic or with the more

stable colouration typical of the remainder of the year. This transitory period of high pigmentation usually occurs between early spring and mid-autumn.

For many species, significant pigmentation of the bark (and leaves) occurred only during this period of new growth but a few species growing at high altitudes e.g. *Epacris gunnii, E.lanuginosa, E.petrophila, Archeria comberi, Richea acerosa, R.gunnii* and *Trochocarpa cunninghamii* consistently produce leaves with dark red pigmentation and this is very marked during winter.

Anthocyanin patterns varied significantly between different tissues but cyanidin derivatives were always present in all cyanic organs examined. Delphinidin derivatives were most frequent in twig bark and fruit, and their occurrence elsewhere (excepting in *Acrotriche serrulata* and *Woollsia pungens*) is thought to be due to contamination from traces of highly coloured bark tissues which remained attached to pedicels and petioles. Pelargonidin derivatives were found only in fruit and floral tissues.

(i) Flowers and Leaves

In general, flowers and leaves exhibited the simplest and most consistent patterns. Only two or occasionally three pigments were detected in significant quantities, and these were restricted to cyanidin derivatives i.e. Cy-3-Gal, Cy-3-Arab and Cy-3-RhaGal. In white flowered species, anthocyanins were derived from red anthers, or from sepals and bracts which were too small to be removed conveniently. Anther pigments exhibited the same general patterns as those from coloured petals.

(ii) Twig bark

Five pigments were present in significant quantities in the bark i.e. Cy-3-Gal, Cy-3-Arab, Cy-3-RhaGal, Dp-3-Gal and Dp-3-Arab. Cy-3-RhaGal was not present as a dominant anthocyanin and usually did not co-occur with Dp-3-Gal and Dp-3-Arab. Anthocyanin glucosides were found in only one species (*Trochocarpa gunnii*) in which Cy-3-RhaGlc was the dominant pigment. A similar pattern would be expected in the twig bark of *T.disticha* since this species, in common with *T.gunnii*, accumulates anthocyanin glucosides rather than galactosides in its fruit.

(iii) Fruit

The greatest complexity, both numerically and structurally, occurs in anthocyanins of the Styphelieae fruit. All anthocyanins, with

the exception of malvidin glycosides, were observed in fruits from this subfamily. However, not all pigments occurred together in any single species.

Anthocyanins present as dominant pigments included Cy-3-Gal, Cy-3-Arab, Cy-3-RhaGal, Cy-3-RhaGlc, Dp-3-RhaGal and Dp-3-RhaGlc. In addition, Cy-3-Glc, Cy-3-Rha, Cy-3-XylGal, Dp-3-Gal and Dp-3-Arab were present in significant quantities in a restricted number of species. Anthocyanins were not detected (or were present at concentrations too low for chromatographic analysis) in the fruits of 15 species i.e. *Acrotriche serrulata, Astroloma humifusum, A.pinifolium, Leucopogon australis, L.collinus, L.ericoides, L.lanceolatus, L.parviflorus, L.stuartii, L.virgatus, Monotoca elliptica, M.empetrifolia, M.glauca, M.scoparia, M.submutica* and *Styphelia adscendens*. Pigmentation of fruits in these species was caused by other flavonoid compounds, carotenoids or chlorophylls.

In the Epacrideae, pigmentation was restricted to cyanidin derivatives and only two anthocyanins, Cy-3-Gal and Cy-3-Arab, occurred as dominant pigments. In general, capsules were only weakly coloured by anthocyanins.

'Flavonoids'

None of the more common 'flavonoids' was found to be tissue-specific but general trends in the relative accumulation of certain pigments, and in the complexity of pigment patterns, were apparent in the different tissues.

(i) Leaves

In general, leaf chromatograms exhibited the most complex patterns and all flavonols considered in this work, excepting Qu- and Km-5-Glc, were found. However, no single species accumulated all compounds. Quercetin derivatives, particularly Qu-3-Gal and Qu-3-Glur, were widespread and frequently dominant.

(ii) Flowers

The concentration of flavonol rhamnosides was conspicuously higher in the flowers than elsewhere and in many species e.g. *Epacris* and *Leucopogon*, these were the dominant glycosides. The occurrence of kaempferol in the flowers (92%) was slightly higher than for the leaves (88%) but that of myricetin was considerably lower (38% in the flowers versus 56% in the leaves).

Most compounds were detected in the flowers but several species-specific (and tissue-specific) compounds were absent e.g. OPi_1

and OPi_2, D_{42}, D_{43}, D_{44}. *Astroloma pinifolium* was the only species in which no flavonols were detected in the flowers and the bright yellow-orange colour is attributed to carotenoid pigments.

(iii) Fruit

The distribution of fruit pigments was determined in the Styphelieae only, and in these tissues, chromatographic patterns were generally the simplest observed. The occurrence of myricetin derivatives was significantly higher than elsewhere i.e. 81% in the fruit versus 38% (flowers) and 56% (leaves). This parallels the more frequent occurrence of delphinidin in the fruits than in other tissues.

Flavonols were not detected in the fruit of three species i.e. *Astroloma pinifolium*, *Trochocarpa disticha* and *T.cunninghamii*.

5. Intraspecific Variation of Pigment Patterns

Probably because of the simplicity of anthocyanin patterns, intraspecific variation was insignificant and was usually restricted to minor constituents. As such, it was considered quantitative in nature. An apparent exception occurred with Cy-3-XylGal in *Cyathodes dealbata* (fruit). Although this pigment was not produced as the dominant anthocyanin, it was always present in significant quantities when detected on 2-D chromatograms. Of the 6 populations examined, it was absent from L.Augusta and showed some variation at Mt.Field. A second pigment, Cy-3-XylArab, showed intra- and inter-population variation in *Leucopogon virgatus*. However, this anthocyanin was always observed as a minor constituent when present and its variation is considered quantitative.

Intra-specific variation was more pronounced among 'flavonoid' patterns but was generally confined to inter-population differences. However, for many species, it was not possible to obtain samples from several populations for this work (see Table A5) and the extent of these differences could not be ascertained completely. Nevertheless, where it was possible to examine several populations, a comparison between chromatographic patterns indicated a range of intra-specific variation among the different species. In some cases it was absent or insignificant but many examples could be cited where variation involved some of the dominant compounds e.g. in *Cyathodes juniperina* (leaves), myricetin derivatives are absent from populations at Tasman Peninsula, in *Epacris tasmanica* (leaves), myricetin derivatives are present in the more northern populations but were not detected in the more southern

populations, in *E.obtusifolia* (flowers), Km-3-Rha was absent from the population at McPartlan Pass (south-west) whilst it was prominent at Bicheno (east), in *Richea pandanifolia* and *R.curtisii*, Qu-3-Glur was present from the Hartz Mts. but was absent elsewhere, and so on. This problem of inter-population variation obviously needs more study for 'flavonoids', and it may be possible to relate variation to ecological differences.

(i) Variation in the glycosidic relationship (G.R.)

With both anthocyanins and flavonols, inter- and intra-population variation of G.R. is negligible. It was apparent in one species only i.e. *Leucopogon stuartii* which accumulated flavonol galactosides to the exclusion of flavonol glucuronides in Tasmanian populations but in New Zealand (i.e. = *Cyathodes fraseri*) it accumulated both glycosidic types with the latter at greater concentrations than the former.

Limited variation in G.R. between tissues within individual species was apparent. With anthocyanins, *Archeria serpyllifolia* and *Epacris obtusifolia* were unusual in having a different G.R. in the leaves and flowers, and with flavonols, variation occurred with *Archeria serpyllifolia, Dracophyllum minimum, Epacris acuminata, Richea milliganii, Sprengelia incarnata, Leucopogon setiger* and *Pentachondra pumila.*

(ii) Variation in the occurrence of Myricetin

In studying the distribution of flavonols, two approaches were taken initially i.e. the crude extract was divided and half was used to determine the presence of glycosides whilst the other half was hydrolysed and tested for the three common aglycones, kaempferol, quercetin and myricetin. In an attempt to avoid as much confusion as possible from contaminants, the aglycone chromatograms were run in two dimensions (see p. A5) as well as the more usual one dimension. However, the examination of aglycones in this manner was not continued throughout the study because it became apparent that a high degree of inaccuracy involving myricetin was present.

A comparison between glycoside and aglycone papers showed a discrepancy between the two with respect to myricetin. One hundred and twenty two aglycone samples were tested and myricetin was detected in 41. With 20 other extracts, whilst myricetin was absent on the aglycone papers, it was recorded as present on the glycoside papers. In 4 of these extracts, myricetin was a minor constituent among the glycosides and its absence on the aglycone papers could not be considered

significant. Nevertheless, there remains almost one quarter of the samples known to contain myricetin which proved negative when the aglycone papers were examined. Such a high degree of error cannot be ignored.

It has already been mentioned (p. 50) that myricetin derivatives appeared more labile during the purification techniques. It seems likely that under the severe conditions encountered during hydrolysis (hot mineral acid), not only is the sugar removed from the aglycone, but the aglycone itself is susceptible to breakdown.

From an examination of published data, it seems likely that this problem is not restricted to my own research.

Twenty seven species from the Epacridaceae were examined by Harborne and Williams (1973) for the occurrence of several leaf flavonols, including myricetin. I have also examined 23 of these species and found myricetin in 10. Harborne and Williams recorded myricetin in 2 species only, and one of these I did not examine. Hence, a total of 9 disagreements with respect to myricetin, in 23 species, occurred between these two studies. In addition to this, Bate-Smith (1962) examined 3 species for myricetin (and other compounds) and found no trace of its presence. However, I have also examined these 3 species and have detected myricetin in all.

The most probable explanation of these discrepancies lies with the lability of myricetin, and this may have been accentuated if Harborne and Williams and Bate-Smith used herbarium material. Alternatively, the differences may be due to intra-specific variation but I feel that this is the least likely explanation.

The work undertaken here suggests that the reliability of myricetin frequency obtained from hydrolysis procedures may be questioned. Bate-Smith (1973) reports that while the occurrence of kaempferol and quercetin is reproducible within species, the same is not true for myricetin. These results would support the view presented here. Hence, it seems that the occurrence of myricetin in the plant kingdom may be more widespread than is generally believed, the apparent rarity being due to its susceptibility to breakdown under the conditions used in its detection.

C. DISCUSSION OF THE CHEMICAL INVESTIGATION

1. General

(a) Anthocyanins

The characteristic anthocyanins of the Epacridaceae i.e. the galactosides and arabinosides, have not been reported outside the Angiosperms but their distribution within this group is relatively widespread, particularly in the Dicots (e.g. the Sterculiaceae, Lythraceae, Rosaceae, Ericaceae, etc. - for the most complete treatment, see Harborne - 1967). In some families e.g. the Ericaceae, the occurrence of galactosides and arabinosides appears to be associated, and Harborne (1967) has suggested that this may be due to the close stereochemical relationship between the two sugars, L-arabinose and D-galactose.

In agreement with their occurrence in the Epacridaceae, Cy-3-Gal and Cy-3-Arab have been reported more frequently than the corresponding pelargonidin, delphinidin or malvidin derivatives which, when present, are usually reported as minor compounds accompanying the more prominent cyanidin pigments. Pelargonidin galactoside and arabinoside appear to be particularly rare and the latter was reported for the first time only relatively recently (Lowry - 1971b). Malvidin galactoside and arabinoside are also rare although both have been reported in the Ericaceae (Francis *et al.* - 1966) and recently the latter has also been found in the Lythraceae (Saleh - 1973) along with Dp-3-Arab and Pet-3-Arab.

Cy-3-Glc and Cy-3-RhaGlc are rare in the Epacridaceae but they are the most common anthocyanin monoside and bioside respectively in the plant kingdom. Both have been isolated from the lower plant groups and their occurrence in the Angiosperms is widespread within both the Monocots and the Dicots (see Harborne - 1967). The pelargonidin and delphinidin glucosides and rhamnosylglucosides are less common but are nevertheless widespread.

Cyanidin and delphinidin 3-rhamnosylgalactosides are the two most common biosides in the Epacridaceae but outside the family they have been reported only in the Cornaceae (Du and Francis - 1973). Although suspected, the presence of Pel-3-RhaGal has not yet been confirmed in the Epacridaceae.

The remaining anthocyanins identified in the Epacridaceae i.e. Cy-3-Rha, Cy-3-XylGal and Cy-3-XylArab are all rare, and the latter pigment, Cy-3-XylArab, has yet to be reported outside the family.

(b) Flavonols

The characteristic flavonols of the Epacridaceae are 3-glucuronides, 3-galactosides and 3-arabinosides. The latter two are known from several plant families e.g. Apocynaceae (Urbatsch and Mabry - 1974), Sapotaceae (Subramanian and Nair - 1971), Harborne and Williams - 1971b), etc., but glucuronides are relatively rare outside the Epacridaceae. Families in which they have been recorded include the Umbelliferae (Harborne and Saleh - 1971), Rosaceae (Ryan and Coffin - 1971), Compositae (Saleh *et al.* - 1971), etc. In general, quercetin derivatives are more common than the analagous kaempferol and myricetin derivatives. Flavonol-3-glucosides, the most common flavonol monosides in the plant kingdom, have not been established conclusively in the Epacridaceae although they probably do occur. On the other hand, flavonol-3-rhamnosides are common both within the family and outside. (For the most comprehensive distributions of these pigments, see Harborne - 1967.)

The most widespread flavonol bioside in the plant kingdom is quercetin 3-rhamnosylglucoside (rutin) and this appears to be consistent in the Epacridaceae. Kaempferol amd myricetin 3-rutinosides occur less frequently. Quercetin 3-xylosylrhamnoside, which is found sporadically in the Epacridaceae, has not been reported outside the family.

Flavonol-5-glucosides, found in one Epacrid species, are also rare outside the family. They have been reported in the Compositae and the Labiatae (Glennie and Harborne - 1971).

2. Taxonomic Implications

(a) Glycosidic relationship

Examination of the anthocyanin G.R. may have some taxonomic application. Of the genera examined, *Archeria, Epacris, Sprengelia, Woollsia, Astroloma, Pentachondra* and *Styphelia* all accumulate arabinosides in excess of galactosides whilst the converse is true for *Richea, Dracophyllum, Cyathodes* (with one exception), *Lissanthe, Monotoca* and *Trochocarpa*. Both relationships are found in *Leucopogon* but it is not possible to ascertain the significance of this because of the low number of species available for study (10/159 spp.). Certainly the genus has been referred to as a composite one (Smith-White - 1955, 1959a, Franks and Watson - 1963) and possibly some future study will result in its division into several smaller groups. In the few species studied, G.R. is consistent at the Series level (see Table 19 - p. 78).

Section	Series	Species	G.R. A	G.R. F
Perojoa	I. Psilostachyae	*L.lanceolatus*	g > a	gr > g
	II. Australes	*L.parviflorus*	g > a	gr > g
		L.australis	g > a	gr > g
	III. Collinae	*L.collinus*	a > g	gr > g
		L.microphyllus	a > g	
	VII. Virgatae	*L.virgatus*	a > g	gr > g
Heteranthesis		*L.hookeri*	g > a	g
		L.maccraei		g
Pleuranthus	II. Ericoides	*L.ericoides*	a > g	gr
	III. Micranthae	*L.esquamatus*		gr > g
	IV. Planifoliae	*L.setiger*	a > g	gr
		L.fraseri	a > g	g
		L.juniperinus		g

Table 19. The relationship between G.R. and Series in some *Leucopogon* species. (A = anthocyanins, F = flavonols, g = galactose, gr = glucuronic acid and a = arabinose)

It is difficult to estimate which glycosidic type is dominant in *Prionotes* and *Brachyloma* because both appear equally concentrated. In *Cyathodes, C.petiolaris* accumulates more arabinosides than galactosides contrary to other species of the genus. However, this species is closely allied to *Leucopogon* and has several characteristics which are typical of that genus (see p. 144). It appears that chemistry is simply reflecting an anomaly already apparent from morphology. In *Acrotriche,* only two species were available and each was characterized by a different relationship. (A similar situation also occurred with flavonol glycosides.) The two differ markedly in habit but both appear to be "good" *Acrotriche* species. At present, I cannot offer any explanation for the variation shown between the two species but possibly an examination of the remaining ten species (all from the Australian mainland) may suggest some reason.

A comparable situation to that observed among the anthocyanins does not appear to operate between flavonol galactosides and arabinosides. With six exceptions (*Epacris crassifolia* - leaves, *E.mucronulata* - flowers, *Woollsia pungens* - leaves, *Richea procera* - leaves, *Acrotriche divaricata* - flowers and *Cyathodes juniperina* - leaves, N.Z.), the concentration of arabinosides never exceeds that of galactosides. Hence, the taxonomic application of this relationship is very limited.

An alternative relationship between flavonol glucuronides and galactosides is apparent but at the generic level, it is less consistent

than the anthocyanin relationship. In addition, the relationship is complicated in the flowers by the presence of dominant rhamnosides. These glycosides may themselves be taxonomically significant at the generic level e.g. in the flowers of *Epacris* and *Leucopogon*, rhamnosides are accumulated in excess of both galactosides and glucuronides in most species.

The relationship between galactosides and glucuronides may have greater taxonomic importance at the sub-generic level. However, representation of the larger genera such as *Acrotriche*, *Astroloma*, *Leucopogon*, etc., is so poor in this work that it is difficult to test the hypothesis. Certainly, as far as the numbers allow, there is some supporting evidence e.g. in *Epacris*, species from group E1 (see p. 118) are characterized by dominant glucuronides whilst in group E2, with the exception of *E. lanuginosa*, glucuronides and galactosides are present in approximately equal concentrations. In *Cyathodes*, species from groups C1 and C3 (see p. 135) are characterized by dominant glucuronides whilst group C2 and *Cyathodes dealbata* are characterized by dominant galactosides. In *Leucopogon*, G.R. is consistent in Sections Perojoa and Heteranthesis but shows some variation in Section Pleuranthus (see Table 19, p. 78). In *Astroloma*, the two species studied show a different glycosidic relationship and each belongs to a different section.

(b) Identification of species

Relatively few species are characterized by species-specific compounds. *Cyathodes dealbata*, *Leucopogon virgatus* and *Richea pandanifolia* accumulate anthocyanins which may be considered species-specific if individual organs are compared, but the relevant compounds are usually minor pigments whose presence shows intra-specific variation. Several flavonols are species-specific, and the number increases if comparable tissues are considered. A list of these compounds, and the species and tissues in which they occur, is given in Table 20 (p. 80).

Chromatographic patterns, particularly those of the flavonols, offer considerably more potential for species identification. Most flavonol patterns are species-specific and should prove helpful in situations where morphological characters are not sufficient to distinguish between species. However, the extent of intra-specific variation has not been ascertained in many species, and may influence the practical application of chromatographic patterns in species identification. Unique anthocyanin patterns are relatively scarce but in conjunction with the G.R., can be used to a limited extent in species identification.

Pigment	species	tissue
Anthocyanins		
Mv-3-Gal, Mv-3-Arab	*Woollsia pungens*	leaves
Cy-3-XylGal	*Richea pandanifolia*	flowers
Mv-3-Gal, Mv-3-Arab	*Woolsia pungens*	flowers
Cy-3-XylArab	*Leucopogon virgatus*	flowers
Cy-3-XylGal, Cy-3-XylArab	*Cyathodes dealbata*	fruit
Flavonols		
D_{42}, D_{43}, D_{44}	*Epacris lanuginosa*	leaves
D_{47}	*E.* var. *Davies Ck.*	leaves
Pc/Black	*Prionotes cerinthoides*	leaves
D_{39}	*Brachyloma depressum*	leaves
D_{26a}, D_{26b}	*Cyathodes juniperina*	leaves
Br_1	*Leucopogon collinus* var. *alpina*	leaves
Ch_1	*L.virgatus*	leaves
D_{18}	*Monotoca submutica*	leaves
Qu-3-XylRha	*Prionotes cerinthoides*	flowers
Qu- and Km-5-Glc	*Richea procera*	flowers
D_{26a}, D_{26b}	*Cyathodes juniperina*	flowers
D_{38}	*Monotoca scoparia*	flowers
D_{50}	*Cyathodes dealbata*	fruit
D_{26a}, D_{26b}	*C.juniperina*	fruit
Br_1	*C.parviflorus*	fruit

Table 20. Species-specific compounds using comparable tissues.

(c) Significance of flavonoids in each genus

General comments regarding the significance of flavonoid compounds in each genus is given below. This work is very superficial, and in most cases, further detailed investigation is required.

ARCHERIA

Four endemic Tasmanian species and one endemic New Zealand species (*A.traversii*) were examined. All five could be identified on the basis of flavonol patterns, but anthocyanin patterns were useful only in the identification of *A.serpyllifolia* (flowers) where the usual G.R. is reversed.

Two species, *A.hirtella* and *A.eriocarpa*, are very similar in vegetative morphology although they can be distinguished when flowers are present. The different chemical patterns in the leaves of these two species should prove valuable in their identification when only vegetative material is available.

DRACOPHYLLUM

Six species were considered, 2 from Tasmania, 3 from New Zealand

and one from New South Wales. The latter species, *D.secundum*, was not examined for flavonols but the remaining 5 appear to be divided into two groups, with *D.milliganii* (Tas.) and *D.uniflorum* (N.Z.) accumulating myricetin derivatives in excess of quercetin derivatives whilst the converse is true of *D.minimum* (Tas.), *D.recurvum* and *D.strictum* (N.Z.)

EPACRIS

Twenty nine species or varieties were considered, 19 from Tasmania, 9 from New South Wales and one from New Zealand. The genus is variable morphologically and species are often difficult to identify.

Morphological and chemical relationships among the Tasmanian species have been discussed in Part III, (p. 116). The genus is consistent with respect to anthocyanin patterns but can be divided into two groups on the presence or absence of flavonol glucuronides. Three of the 8 species lacking flavonol glucuronides are united by the presence of 2 prominent, but unidentified, dihydroflavones i.e. *E.impressa* (Tas.), *E.pulchella* (N.S.W.) and *E.reclinata* (N.S.W.). Detailed morphological comparisons between these three have yet to be undertaken.

The identity of *Epacris* var. *N.S.W.* (see p. C9) is unknown and it may be a new species. From vegetative morphology and ecological evidence, it was thought that this plant might have been a variety of *E.obtusifolia*. However, chemical evidence would not support this relationship.

E. var. *Davies Ck.* and *E.lanuginosa* are the only two species examined which accumulate species-specific compounds. With the latter species, these were consistent in every population examined and serve to distinguish it immediately from the closely related and morphologically similar *E.paludosa* (Bass Strait Is., N.S.W.).

LEBETANTHUS, PRIONOTES

These two monotypic genera are endemic, the former to South America and the latter to Tasmania. Floral tissue was not available for the examination of *Lebetanthus*.

The two species do not appear particularly close with respect to flavonol chemistry, and there is no evidence to suggest that they should be included in a single genus, nor that they should form a tribe, subfamily or family separate from other members of the Epacridaceae. They have a different G.R. in their leaves with *Prionotes* accumulating glucuronides in excess of galactosides whilst in *Lebetanthus*, galactosides are dominant and glucuronides appear to be completely absent. The accumulation of D_{34} and the absence of Qu-3-XylRha and Pc/Black in

Lebetanthus places this genus chemically closer to *Epacris* than to *Prionotes*.

RICHEA

Ten species or varieties were examined, 9 endemic to Tasmania and one, *R.continentis*, from New South Wales (and Victoria). From morphological characters, one species, *R.curtisii*, is probably a hybrid between *R.pandanifolia* and *R.scoparia*. In native populations, the plant occurs sporadically, and I have not seen more than two or three plants in any population. At each location (Mt.Field, Hartz Mts., Adamsons Peak), it is closely associated with *R.scoparia*, with *R.pandanifolia* being nearby. Leaf flavonols were available for comparison in these three species, and these do not conflict with the possibility of hybridization. All three species show intra-specific variation, and comparative results from two populations are shown below in Table 21.

	Flavonols
Hartz Mts.	A B C D E F G H
R.pandanifolia	● ⊘ o + +
R.scoparia	● o + + ⊘ +
R.curtisii	● ⊘ o o + o +
Eagle Tarn (Mt.Field)	
R.pandanifolia	● o o ⊘
R.scoparia	● o o + ⊘ o +
R.curtisii	● o o + ⊘

Table 21. Comparison of flavonls in leaves of *R.pandanifolia*, *R.scoparia* and *R.curtisii* from two populations.

(A = Qu-3-Gal, B = Qu-3-Glur, C = Qu-3-Arab, D = Qu-3-Rha, E = Km-3-Rha, F = Km-3-Arab, G = My-3-Gal, H = D_{50}. The order of pigment concentration is ● > ⊘ > o > +)

No rigorous studies have been undertaken as yet to test the hypothesis of hybridization.

Species-specific compounds are present in *R.procera* but these are accumulated only in the anthers and operculum. Chromatographic patterns of leaf flavonols are of greater practical use and serve to distinguish this species from *R.sprengelioides* which, in several localities, is very similar in its vegetative morphology.

SPRENGELIA

Only one species of *Sprengelia* was examined i.e. *S.incarnata*. It was unusual in having a different G.R. among the flavonols of the leaves

and flowers. Further studies are required to investigate the extreme morphological variation which occurs between upland and lowland forms of this species.

WOOLLSIA

This monotypic genus is not represented in Tasmania but is endemic to New South Wales. *W.pungens* differed from all other species examined in containing methylated anthocyanins (malvidin derivatives) and in accumulating flavonol arabinosides as the dominant flavonol glycosides.

Species from the closely related genus, *Lysinema*, from Western Australia were not available for comparison.

ACROTRICHE

Two species of *Acrotriche* were examined, *A.serrulata* (Tas., Vic., N.S.W.) and *A.divaricata* (N.S.W.). The two species differed in their glycosidic relationship in both anthocyanins and flavonols but it is possible that in the latter pigments this is not significant (see p.58).

An investigation into the morphological variants of *A.serrulata* in Tasmania has yet to be undertaken.

ASTROLOMA

Two Tasmanian species were available for comparison. The glycosidic relatioship between the major flavonols differed in the two, suggesting that an examination of additional species would be valuable. The chemical differences observed appear to be reflecting differences in cytology (see Smith-White - 1955) as well as morphology (the two are placed in different sections of the genus).

BRACHYLOMA

Two Tasmanian species and one species collected from New South Wales (*B.daphnoides*) were examined. The chemical results obtained were very uniform suggesting a closely related group. However, I have not yet seen the fruit of any of these species, nor the flowers of *B.depressum*.

CYATHODES

Twelve species or varieties of *Cyathodes* were examined, one of which was endemic to New Zealand (*C.empetrifolia*). Two additional species from New Zealand i.e. *C.colensoi* and *C.fraseri* are placed in the genus *Leucopogon* in Tasmania (i.e. *L.hookeri* and *L.stuartii* respectively). The absence of myricetin in both species does not support their inclusion in *Cyathodes*. Furthermore, *C.colensoi* does not accumulate flavonol glucuronides in accordance with most other *Cyathodes*, whilst *C.fraseri* is unusual in not accumulating Qu-3-Arab. From morphological

characters, and the limited chemical information obtained here, it seems preferable to retain both species in the genus *Leucopogon*. A third species from New Zealand, *C.fasciculata*, does not occur in Tasmania but prior to 1960, it was also included in the genus *Leucopogon* (see Cheeseman - 1906). From the chemical results obtained here, it would fit satisfactorily into either genus but in common with the preceeding two New Zealand accessions, it has been considered in the genus *Leucopogon* in this thesis.

Tasmanian species of *Cyathodes* are discussed in detail in Part III.

LEUCOPOGON

Seventeen species or varieties were available for examination, six being obtained from the mainland and three from New Zealand (received as *C.fraseri*, *C.colensoi* and *C.fasciculata* - see above).

On the basis of chemical data, there is no evidence to support the separation of *Leucopogon ericoides* var.*coastal* (see p. C9) from *L.ericoides*. (The chemical patterns of these two are virtually the same.) On the other hand, the distinct flavonoid pattern of *L.collinus* var. *alpina* would serve to distinguish it immediately from *L.collinus*. This plant is probably *L.ciliatus* var. *minor* Hook. which is normally included in *L.collinus*. It is unusual in the genus in accumulating My-3-Rha as the dominant leaf pigment.

LISSANTHE

Two Tasmanian species (both represented on the mainland) and one New South Wales species were examined. One of these, *L.montana*, is morphologically similar to *Leucopogon hookeri* but the two differ in the degree of hairiness of the corolla lobes, the former having glabrous lobes whilst the latter has densely hairy lobes. However, populations of both species from Tasmania's Central Plateau indicate that overlapping variation is apparent with respect to this character.

While keeping the two species separate, Willis (1956) has removed *Lissanthe montana* to the genus Leucopogon, i.e. *L.montanus*. There can be no doubt that *Lissanthe montana* is chemically consistent with the two species, *Leucopogon hookeri* and *L.maccraei*, examined from the Section Heteranthesis of the genus *Leucopogon*. However, the removal of *Lissanthe montana* on the basis of its chemical data would be premature at this stage, particularly in view of the small number of species examined from Section Heteranthesis and the chemically consistent nature of the genus *Lissanthe*.

MONOTOCA

Nine species or varieties of this genus were examined, all from Tasmania. Problems encountered in the genus are discussed in detail in Part III, p. 148.

PENTACHONDRA

Three species were available for study, one being common to Tasmania and New South Wales (*P.pumila*) whilst the remaining two are endemic to Tasmania.

Chemical data would suggest that *P.involucrata* and *P.ericaefolia* are closer to each other than to *P.pumila*, the former two having dominant flavonol galactosides whilst the latter has dominant glucuronides.

STYPHELIA

Four species were studied, three collected from New South Wales whilst the fourth was collected in Tasmania. All four species are chemically consistent.

TROCHOCARPA

Five species of *Trochocarpa* were included in the survey, four being endemic to Tasmania whilst the fifth, *T.laurina*, was obtained from New South Wales.

Two species, *T.gunnii* and *T.disticha*, are unusual in producing anthocyanins containing glucose rather than galactose. One of these, *T.disticha*, is morphologically similar to *T.cunninghamii*, and in the past, has sometimes been considered as a variety of this species (Bentham - 1869, Rodway - 1903). *T.cunninghamii* and *T.disticha* differ chemically in their incorporation of either glucose (*T.disticha*) or galactose (*T.cunninghamii*), but not both into their anthocyanins. Unfortunately, it is not possible to use this knowledge for routine identification purposes because R_fs of the relevant anthocyanins are so similar that they cannot be differentiated easily on 2-D chromatograms.

(d) Contribution towards classification problems

The flavonoid data obtained in this work does not support the need for a new classification but it does assist in choosing between some of the existing classifications for the family.

(i) The classification of Bentham and Hooker (1976)

The results obtained here do not conflict with Bentham and Hooker's scheme (see p. 7) and in many genera, supporting evidence can be found (e.g. see arguments in (ii) and (iii) below). However, on

the basis of chemical evidence, it is not possible to discriminate between this classification and that of Watson's. The two differ only in the status of generic groups and not in the delimitation of genera, and even when critical mainland genera such as *Needhamia, Oligarrhena* and *Cosmelia*, etc. become available, their chemical differences may not be pronounced enough to resolve the situation.

(ii) The classification of von Mueller (1867)

The chemical results do not support von Mueller's classification (see p. 7), either in the delimitation of the genus *Styphelia* or the inclusion of the two genera, *Pentachondra* and *Archeria*, into *Trochocarpa* and *Epacris*, respectively.

Seven of the ten genera which von Mueller included in the single genus *Styphelia*, were examined in this work. With anthocyanins, *Acrotriche, Astroloma* and *Styphelia* are consistent in accumulating arabinosides in excess of galactosides whilst the converse is true for *Cyathodes* and *Lissanthe*. Both relationships are apparent in the variable genus *Leucopogon* and the situation is not clear in *Brachyloma*. With flavonol glycosides, *Styphelia* is immediately distinguished as the only genus to accumulate glucuronides and rhamnosides to the exclusion of all other glycosidic types. On the other hand, *Brachyloma* and *Lissanthe* completely lack glucuronides. The four remaining genera, *Acrotriche, Astroloma, Cyathodes* and *Leucopogon*, accumulate both galactosides and glucuronides but are internally variable. From an examination of aglycones, *Cyathodes* differs from the other genera in its high frequency of myricetin, whilst *Brachyloma* and *Astroloma* differ in their relatively low accumulation of kaempferol.

With respect to *Pentachondra* and *Trochocarpa*, the two genera are closely related morphologically and this is apparent in flavonol chemistry, with biosides and myricetin derivatives common in both. However, in anthocyanins, G.R. differs in the two and delphinidin biosides are dominant in *Trochocarpa* but are absent in *Pentachondra*.

The situation is not so clear between *Archeria* and *Epacris* but the chemical differences that are apparent, in conjunction with morphological differences would not support the union of the two. With flavonols, myricetin derivatives are relatively common in *Epacris* but are completely absent from *Archeria* whilst Km-3-Arab and flavonol biosides are rare in *Epacris* but are common in *Archeria*.

(iii) The classification of Drude (1889)

Drude's classification differs essentially from earlier

classifications in the separation of *Prionotes* and *Lebetanthus* into a new tribe, the Prionotae. Chemical results do not support this treatment of the two genera (see p. 82) and their flavonoid patterns are not unusual in the context of the subfamily Epacrideae or indeed, of the whole family.

(iv) The classification of Watson (1967)

It is difficult to evaluate Watson's classification (see p. 8) because several of the critical mainland genera were not examined. There is no chemical support for the removal of *Sprengelia* to a new subfamily, but at the same time, there is some limited support for the subfamily Richioideae. In the two genera studied i.e. *Dracophyllum* and *Richea*, anthocyanin galactosides were accumulated in excess of anthocyanin arabinosides. In all other genera studied from Bentham's subfamily Epacrideae, the reverse G.R. was observed.

(v) The classification of Hutchinson (1973)

Chemical evidence does not support Hutchinson's view that *Prionotes* and *Lebetanthus* should be removed from the Epacridaceae. *Prionotes* accumulates only one compound, Pc/Black, not found elsewhere in the family whilst none was unique in *Lebetanthus*. (See also (iii), above).

3. Phylogenetic Aspects

From the examination of flavonoid compounds, it is possible to speculate upon the evolution of certain groups within the Epacridaceae, and indeed upon the evolutionary status of the compounds themselves.

In the following discussion, the terms 'primitive' and 'advanced' are used to indicate the relative origin and degree of specialization of the various taxa or characters being considered.

(a) The Epacridaceae

Bate-Smith (1962) suggested an irreversible evolutionary relationship, ab → a_ob_o, where a = presence of leucoanthocyanidins, a_o = their absence, b = the presence of tri-hydroxylated compounds (myricetin, leucodelphinidin, ellagic acid, etc.) and b_o = their absence.

In the Epacridaceae, leucoanthocyanidins are present in all species tested and tri-hydroxylated compounds i.e. leucodelphinidin, are common. Myricetin is also common but it may represent a special case (see p. 95). Hence, in this context, the Epacridaceae appears to be a primitive family.

(b) The Epacrideae and the Styphelieae

The occurrence of flavonoid compounds can be related to the proposed evolutionary status of the two subfamilies (see p. 12). Whilst the occurrence of leucodelphinidin is similar in the two groups, there is a conspicuous difference in the flowers, with 47% accumulating this compound in the Epacrideae but only 27% in the Styphelieae. Consequently, of the two, the latter subfamily falls more readily into Bate-Smith's derived category, ab_o. This view is supported by other chemical evidence i.e. the number and complexity of compounds is greater in the Styphelieae than in the Epacrideae, and the diversity of flavonoid patterns and the occurrence of rarer compounds is generally higher. Assuming that structural diversity and complexity of flavonoid compounds is indicative of evolutionary advancement, the results would suggest that the Styphelieae is more advanced than the Epacrideae. A similar impression has been derived from cytological work (Smith-White - 1948) and morphological studies (Paterson - 1961).

(c) Glycosides

In Table 22 (p. 89), a comparison between chromosome number, pollen type, G.R. (anthocyanins), occurrence of galactosides and/or glucuronides (flavonols), breeding system and fruit pigmentation has been shown for some species from the subfamily Styphelieae. Cytological data was obtained from work by Smith-White (1948, 1955, 1959a - see also pp. 12 - 16) and to a lesser extent by Venkata Rao (1961). The pollen of *Cyathodes parvifolia* is listed as T-type even though Smith-White (1955) recorded S-type pollen. This is because Venkata Rao has recorded T-type pollen and from a very brief examination of the species, I have also observed T-type pollen. Other information in the table not recorded by Smith-White, but given by Venkata Rao, includes the haploid number, n = 12, for *Monotoca linifolia* and pollen types for *Trochocarpa cunninghamii* (T-type) and *T.gunnii* (T-type).

In Table 22, the chromosome number and pollen type was not determined in the actual specimen from which the chemical data was obtained but it is assumed that the former characters are consistent within any plant species. The only known exception recorded is *Leucopogon virgatus* with chromosome numbers n = 6 and 10, and S- and T-type pollen respectively. Venkata Rao recorded only S-type pollen from this species in Tasmania (the chromosome number is not given) but presumably if enough populations were examined, the same cytological variation would be apparent. The anthocyanin G.R. for *Leucopogon*

Species	n	PT	An	Fl	BS	FP
Acrotriche divaricata	9	T	g > a	Gal		+
A.serrulata	9	T	a > g	Glur+Gal	H	-
Astroloma humifusum	12	S	a > g	Glur+Gal	H	-
A.pinifolium	7	V		Gal	H	-
Brachyloma daphnoides	9	T		Gal	H	-
Cyathodes dealbata		V	g > a	Gal	H	+
C.divaricata	12	T	g > a	Glur+Gal	D	+
C.glauca	12	T	g > a	Glur+Gal	D	+
C.juniperina	10	T	g > a	Glur+Gal	D	+
C.parvifolia	10	T	g > a	Glur+Gal	D	+
C.petiolaris	9	T	a > g	Glur+Gal	H	+
Leucopogon australis	12	S	g > a	Glur+Gal	H	-
L.ericoides	6	S	a > g	Glur	H	-
L.esquamatus	4	S		Glur	H	
L.hookeri	14	T	g > a	Gal	D	+
L.lanceolatus	24	S	g > a	Glur+Gal	H	-
L.microphyllus	6	S	a > g		H	-
L.parviflorus	6	S	g > a	Glur+Gal	H	-
L.setiger	4	S	a > g	Glur	H	
L.stuartii	4	S	a > g	Gal	H	-
L.virgatus	6	S	a > g	Glur+Gal	H	-
	10	T				
Lissanthe montana	14	T	g > a	Gal	D	+
L.strigosa	7	T	g > a	Gal	H	-
L.sapida	7	T	g > a	Gal	H	+
Monotoca elliptica	12		g > a	Glur+Gal	D	-
M.empetrifolia	12	S	g > a	Gal	D	-
M.linifolia	12	S	g > a	Glur+Gal	D	+
M.scoparia	12	S	g > a	Gal	D	-
Pentachondra involucrata	12	P	a > g	Gal	H	
P.pumila	14	T	a > g	Glur+Gal	H	+
Styphelia adscendens	4	S	a > g	Glur	H	-
S.tubiflora	4	S	a > g	Glur	H	
Trochocarpa cunninghamii		T	g > a	Glur+Gal	H	+
T.gunnii		T	g*> a	Glur+Gal	H	+
T.laurina	10	T		Gal		
T.thymifolia		T	g > a	Gal	H	+

Table 22. A comparison between chromosome number, pollen type, anthocyanin G.R., the occurrence of flavonol glycosides, breeding system and fruit pigmentation (anthocyanin).

(Abbreviations:
n = haploid chromosome number
PT = pollen type - the symbolism used follows that on p. 14.
An = anthocyanin G.R. - a = Arab, g = Gal
Fl = occurrence of flavonol glycosides
BS = breeding system - H = hermaphrodiric, D = dioecious
FP = occurrence of anthocyanin pigmentation in the fruit.)

* The hexose present is glucose but it is grouped with galactose.

virgatus was quite consistent in Tasmania (in 21 populations examined) and showed no variation in the 4 Victorian populations. Only 2 populations were examined for flavonols, but G.R. was consistent in both. Fruit pigmentation and breeding system were determined from actual field observations, or for some mainland species, from fresh cut material.

In Table 23, the information from Table 22 is summarized relating all characters to chromosome number. Data for *Leucopogon virgatus* has not been included in Table 23 because variation in its cytological characters warrants its treatment as two species. The number of species under consideration is small and trends apparent in the table are tentative at this stage, and very speculative.

character	state	chromosome numbers (n) 4	6	12	24	7	9	10	14	spp. no.
pollen type	S	::.	:.	::.	.					27
	T			..		..	::	:.	..	
anthocyanin G.R.	Arab > Gal	::	..	..			..		.	26
	Gal > Arab		.	:::	.	..	.	..	..	
flavonol glycosides Gal &/or Glur	Glur	::	.							26
	Glur+Gal		.	::.	.		..	..		
	Gal	.		:.		:.	.		..	
breeding system	H	::.	..	::	.	.	..			24
	D			::.				..	..	
anthocyanin pigmentation (fruit)	-	..	:.	::.	.	..	:.			25
	+			:.		.	.	..	..	

Table 23. The relationship between chromosome numbers, and pollen type, flavonoid glycosides, breeding system and fruit pigmentation (taken from Table 22.). (• represents a single species)

Smith-White (1955, 1959a,b) has argued that the haploid numbers, n = 4 and 6, are the base numbers for the Styphelieae and consequently their occurrence may be considered representative of the primitive condition. He found a marked association between n = 4 and 6 and their multiples with S-type pollen, and between aneuploid numbers and T-type

pollen. This along with other evidence (see pp. 15 and 16) has led Smith-White to postulate that S-type pollen is more primitive than T-type pollen in the Styphelieae.

Similar reasoning may also be applied to glycoside characters. From Table 23, it is apparent that with anthocyanins, species with the G.R., Gal > Arab are not associated with the basic chromosome numbers, 4 and 6. On the other hand, the G.R., Arab > Gal, is common in association with these numbers even though it is also present in association with the higher numbers. Consequently, this would suggest that Arab > Gal represents the primitive G.R. in the Styphelieae. The accumulation of arabinosides in excess of galactosides was not upset by the advent of polyploidy or aneuploidy, but the reverse relationship, Gal > Arab, may have arisen at the same time or shortly afterwards, possibly as an indirect result of the chromosome change. As the family has evolved, there has been a trend towards the selection of Gal > Arab.

Limited evidence to support the evolutionary status of the 2 character states of G.R. (anthocyanins) is available from an examination of Tasmanian representatives of the Ericaceae (the less specialized family) which are characterized by the G.R., Arab > Gal. (However, only 4 species of Ericaceae are present in Tasmania and only 3 were available for chemical comparisons.) In addition, a comparison of G.R. between the two subfamilies of the Epacridaceae indicates that Arab > Gal is more widespread in the Epacrideae (thought to be the more primitive subfamily) than in the Styphelieae whilst the converse is true for Gal > Arab. Furthermore, although Cy-3-Arab is present in all species of the Styphelieae, in particular tissues of several species it is either absent or accumulated in extremely minor quantities. This would suggest a tendency in the more advanced subfamily to reduce the concentration of arabinosides.

A similar trend to that described for anthocyanins is also apparent in the flavonols. For clarity, the results in Table 23 are shown in Table 24 with some slight re-arrangements (p. 92).

From this table it is apparent that the occurrence of flavonol glucuronides (unaccompanied by flavonol galactosides) are closely associated with the basic chromosome numbers, n = 4 and 6, while at the other extreme, flavonol galactosides (unaccompanied by glucuronides) are mostly associated with polyploid and aneuploid numbers. Thus, the results suggest that the accumulation of glucuronides represents a primitive characteristic in the Styphelieae. The occurrence of

flavonol glycosides	chromosome group basic	polyploid	aneuploid
Gal	1	3	6
Glur + Gal	1	6	4
Glur	5	0	0

Table 24. Comparison between chromosome number and occurrence of flavonol galactosides and glucuronides.

galactosides and glucuronides is not mutually exclusive, and an intermediate condition between the two extremes exists where both glycosidic types are represented. In an analagous situation to that described for the anthocyanins, the accumulation of glucuronides appeared unaffected by the advent of polyploidy and aneuploidy. However, the ability to accumulate galactosides also occurred about this time and was selected for in preference to glucuronides. Consequently, there has been a trend towards greater utilization of galactose in the Styphelieae.

In relating fruit pigmentation to chromosome numbers, it is apparent that anthocyanin colouration is associated only with polyploidy and aneuploidy. Using similar arguments to those above, one may postulate that anthocyanin pigmentation of fruits represents an advanced condition over pigmentation by chlorophylls and carotenoids.

An examination of the breeding system is also of interest. Hutchinson (1973) has suggested that a dioecious breeding system is more advanced than a hermaphroditic one. Data shown in Table 23 would also suggest this evolutionary relationship i.e. no species having the primitive chromosome numbers is dioecious, but 7 are hermaphroditic.

The suggested evolutionary status for the various states of the 6 characters considered here are summarized in Table 25.

By ascribing the values 1, ½ and 0 to advanced, intermediate and primitive characters respectively, it is possible to quantitate the data in Table 22. In this manner, an evolutionary index (E.I.) can be calculated indicating the relative degree of specialization occurring among species of the table with respect to the 6 characters used (Table 26).

character	primitive	intermediate	advanced
chromosome number	4,6		12 24 7 9 10 14
pollen type	S	V, P	T
anthocyanin G.R.	Arab > Gal		Gal > Arab
flavonols (Gal vs. Glur)	Glur	Glur+Gal	Gal
breeding system	H		D
fruit anthocyanins	-		+

Table 25. Suggested evolutionary status of 6 characters in the Epacridaceae.

Species	M	n	PT	An	Fl	BS	FP	E.I.
Acrotriche divaricata	+	A	A	A	A		A	≥ 5.0
A.serrulata	+	A	A	P	I	P	P	2.5
Astroloma humifusum	–	A	P	P	I	P	P	1.5
A.pinifolium	–	A	I		A	P	P	≥ 2.5, ≤ 3.5
Brachyloma daphnoides	–	A	A		A	P	P	≥ 3.0, ≤ 4.0
Cyathodes dealbata	+		I	A	A	P	A	≥ 3.5, ≤ 4.5
C.divaricata	+	A	A	A	I	A	A	5.5
C.glauca	+	A	A	A	I	P	A	4.5
C.juniperina	+	A	A	A	I	A	A	5.5
C.parvifolia	+	A	A	A	I	A	A	5.5
C.petiolaris	+	A	A	P	I	P	A	3.5
Leucopogon australis	–	A	P	A	I	P	P	2.5
L.ericoides	+	P	P	P	P	P	P	0.0
L.esquamatus	–	P	P		P	P		≥ 0.0, ≤ 2.0
L.hookeri	–	A	A	A	A	A	A	6.0
L.lanceolatus	+	A	P	A	I	P	P	2.5
L.microphyllus	+*	P	P	P		P	P	≥ 0.0, ≤ 1.0
L.parviflorus	+	P	P	A	I	P	P	1.5
L.setiger	–	P	P	P	P	P		≥ 0.0, ≤ 1.0
L.stuartii	–	P	P	P	A	P	P	1.0
L.virgatus	–	P	P	P	I	P	P	0.5
		A	A					2.5
Lissanthe montana	+	A	A	A	A	A	A	6.0
L.sapida	–	A	A	A	A	P	A	5.0
L.strigosa	–	A	A	A	A	P	P	4.0
Monotoca elliptica	+	A		A	I	A	P	≥ 3.5, ≤ 4.5
M.empetrifolia	+	A	P	A	A	A	P	4.0
M.linifolia	+	A	P	A	I	A	A	4.5
M.scoparia	+	A	P		A	A	P	≥ 3.0, ≤ 4.0
Pentachondra involucrata	–	A	I	P	A	P		≥ 2.5, ≤ 3.5
P.pumila	+		A	P	I	P	A	≥ 2.5, ≤ 3.5
Styphelia adscendens	+	P	P	P	P	P	P	0.0
S.tubiflora	–	P	P	P	P	P		≥ 0.0, ≤ 1.0
Trochocarpa cunninghamii	+		A	A	I	P	A	≥ 3.5, ≤ 4.5
T.gunnii	+		A	A	I	P	A	≥ 3.5, ≤ 4.5
T.laurina	–	A	A		A			≥ 3.0, ≤ 6.0
T.thymifolia	+		A	A	A	P	A	≥ 4.0, ≤ 5.0

Table 26. Comparisons of the evolutionary status for the six characters in Table 22, p. 89 , and an estimate of the evolutionary index (E.I.). The occurrence of myricetin in the leaves is also included for comparison against the E.I.

(Evolutionary status: A = advanced, I = intermediate, P = primitive. Characters: M = occurrence of myricetin, n = haploid chromosome number, An = anthocyanin G.R., Fl = flavonol glycosides, BS = breeding system, FP = anthocyanin pigmentation in the fruit.

* Result taken from Harborne and Williams - 1973.)

Although the individual E.I. values for each species must be viewed cautiously, the generic trends are perhaps more meaningful. In Table 27, the range of E.I. values for each genus is given.

Genus	E.I.
Acrotriche	2.5 - 5.0 (6.0)
Astroloma	1.5 - 2.5 (3.5)
Brachyloma	3.5 - 3.0 (4.0)
Cyathodes	3.5 - 5.5
Leucopogon	0.0 - 2.5 (excepting *L.hookeri* = 6)
Lissanthe	4.0 - 6.0
Monotoca	3.0 - 4.5
Pentachondra	2.5 (3.5)
Styphelia	0.0 (1.0)
Trochocarpa	3.0 - 4.0 (6.0)

Table 27. The range of E.I. values for 9 genera from Table 26 (p. 93). (x) = maximum value possible, x = E.I. value based on incomplete information.

An inspection of Table 27 shows that E.I. values are not haphazard within individual genera, but tend to be concentrated over a limited range. This allows the genera to be arranged according to a broad category of evolutionary status i.e. primitive, intermediate and advanced (Table 28).

Primitive	Intermediate	Advanced
Styphelia	← *Pentachondra*	*Cyathodes*
Astroloma	*Brachyloma*	*Lissanthe*
Leucopogon	*Acrotriche*	*Leucopogon hookeri*
	Monotoca	
	Trochocarpa →	

Table 28. Suggested evolutionary status of 9 genera from the subfamily Styphelieae with respect to 6 characters.

The evolutionary status assigned to genera in Table 28 is applicable only to the 6 characters investigated, and since the status of the characters themselves is tentative, the highly speculative nature of the results cannot be over emphasized. Consequently, it would seem advisable not to extrapolate the trends beyond the narrow framework within which the study was undertaken. Nevertheless, the results demonstrate that an integrated investigation of characters from different fields, in this case chemistry and cytology, is a potentially valuable approach to the study of phylogeny.

(d) Myricetin

In the Epacridaceae, it is difficult to reconcile the data with the view that myricetin accumulation is a primitive characteristic while its absence is advanced i.e. $b \rightarrow b_o$ (Bate-Smith - 1962, Harborne - 1967 - in the leaves). Of the two subfamilies, the Epacrideae is considered the more primitive (see pp. 12, 88) and in this subfamily, the occurrence of myricetin in the leaves is 49% and in the flowers, 33% (see Table 12, p. 60). Comparable figures in the more advanced Styphelieae are 62% (leaves) and 41% (flowers). A very high frequency (82%) occurs within the Styphelieae fruits but comparable data is not available for the Epacrideae. (The trend with respect to leucodelphinidin does not appear to be associated with that of myricetin - see Table A6, p. A34 - in accordance with Bate-Smith's $b \rightarrow b_o$ relationship and in fact, in the flowers, it is reversed in the two subfamilies i.e. 27% in the Styphelieae and 47% in the Epacrideae.)

In addition to this, several species with advanced characters (see Table 26, p. 93) are associated with the accumulation of myricetin. In Table 26, 36 species have been assigned an evolutionary index on the basis of 6 characters, and the occurrence of myricetin has been included for comparison. The results are summarized in Table 29.

E.I. (Max. = 6.0)	occurrence of myricetin present	absent
< 3.5	7	
≥ 3.5	14	
$\geq 3.0, \leq 4.0$	1	
≤ 3.5		9
> 3.5		3
$\geq 3.0, \leq 6.0$		2

Table 29. Comparison between E.I. and the occurrence of myricetin in the leaves of 33 species from the Styphelieae.

Fourteen species in this table are without myricetin, and in nine of these, E.I. is ≤ 3.5 whereas for only three species is the E.I. > 3.5 suggesting that absence of this compound is associated with primitive taxa. Of the remaining twenty two species which do accumulate myricetin, seven have an E.I. < 3.5 whereas fourteen species have an E.I. ≥ 3.5. Hence, the trend is towards accumulation of myricetin in the more advanced taxa, rather than in the more primitive ones.

Because the derivation of E.I. values in Table 26 is based on a very low number of characters whose evolutionary status is very

tentative, the trend apparent in Table 29 cannot be considered too meaningful at this stage. However, because the trend is contrary to that frequently accepted for myricetin, the results cannot be ignored and an investigation must be carried further until the situation becomes clear.

4. General comparison between the Epacridaceae and the Ericaceae

A comparison of flavonoid compounds in the Epacridaceae and those reported for the Ericaceae reveals relatively few similarities and some marked differences.

With respect to anthocyanins, the two families are linked by the common occurrence of Cy-3-Gal and Cy-3-Arab. In addition, the pattern of pigmentation in three Tasmanian Ericaceae species (*Pernettya tasmanica, Gaultheria hispida* and *G.depressa*) is consistent with the simpler Epacrid patterns i.e. in *Epacris, Sprengelia,* etc. However, very marked differences are apparent when Epacrid species are compared with Ericaceae species from other continents. All anthocyanidins have been reported in the Ericaceae with the exception of pelargonidin* (Harborne - 1967) whereas in the Epacridaceae, pelargonidin derivatives are present (as minor constituents) and only one methylated anthocyanidin (in one species) was detected. *Styphelia tubiflora* has been re-examined but the earlier claim (Gascoigne *et al.* - 1948) that "partially methylated delphinidin glycosido-acylated derivatives" are present in this plant was not confirmed.

Other differences between the Ericaceae and Epacridaceae include the presence of xylose, rhamnose, galactose, arabinose and glucose in Epacrid anthocyanins, whilst only the latter three sugars are reported from the Ericaceae. In addition, 3-biosides occur commonly in the Epacridaceae, whereas these have been reported infrequently and only in minor quantities in the Ericaceae (Harborne - 1967). Finally, 5-substituted anthocyanins, commonly found in the Ericaceae, and acylated anthocyanins have not been found in the Epacridaceae. From these observations, it is apparent that evolution of anthocyanin pigments has progressed in two separate directions in the two families. The Ericaceae has evolved more complex aglycones but has retained a simple pattern of monoglycoside substitution at position 3, or positions 3 and 5. In contrast, less complex aglycones (non-methylated) have persisted in the Epacridaceae and glycosidic patterns have evolved in

* Pelargonidin is now known to occur in some *Erica* spp. (R.K.Crowden - personal communication).

which 5-substitution appears to play no part but the range of sugars involved in 3-glycosylation has increased and includes more complex sugars (biosides).

The two families accumulate three flavonol aglycones in common i.e. kaempferol, quercetin and myricetin, and possibly a fourth, isorhamnetin. (The identity of the latter aglycone has yet to be confirmed in the Epacridaceae - see p. 53.) In the Epacridaceae, quercetin, kaempferol and myricetin occur in the leaves of 99%, 88% and 56% of the species respectively whilst comparable figures for the Ericaceae are 100%, 33% and 45% (calculated from the work of Harborne and Williams - 1973) i.e. the occurrence of both kaempferol and myricetin is higher in the Epacridaceae and their relative accumulation is reversed compared to the Ericaceae.

Both families accumulate leucoanthocyanidins and dihydroflavones but the structure of the latter pigments is still in doubt in the Epacridaceae. The presence of 8-hydroxylated aglycones (gossypetin), 5-methylated aglycones (azealetin, 5-OMe-myricetin) and 3,5-methylated aglycones (caryatin) have been reported in the Ericaceae (Harborne and Williams - 1971) but none of these were detected in the Epacridaceae. Instead, in the latter family, 5-glycosylation was apparent although it was rare.

Flavonol-3-galactosides, 3-arabinosides and 3-rhamnosides have been identified in both families. However, the identity of Qu-3-Arab from the Epacridaceae appears to be different from Qu-3-Arab obtained from the Ericaceae (see p. 51). Flavonol 3-glucuronides, widespread throughout the Epacridaceae, have been reported infrequently in the Ericaceae (Sasaki and Watanabe - cited in Harborne, 1967). At least eight 3-biosides have been detected in the Epacridaceae but none of these has been reported from the Ericaceae although two others, azealetin 3-RhaGlc (De Loose - 1969) and My-3-GalGal (Puski and Francis - 1967) are known.

The results obtained for flavonols support the differences apparent in anthocyanin studies i.e. that diversification in the Ericaceae has been directed towards greater complexity in aglycones (methylation, 8-hydroxylation) whereas in the Epacridaceae, greater variation in glycosylation has evolved.

A general comparison of flavonoid constituents in the two families is given in Table 30.

Pigments	Epacridaceae	Ericaceae
Anthocyanins		
cyanidin, delphinidin	+	+
pelargonidin	rare	rare
malvidin	rare	+
peonidin, petunidin	-	+
galactose, arabinose	+	+
rhamnose	+	-
xylose (biosides)	+	-
3-biosides	+	rare
3,5-diglycosylation	-	+
acylation	-	+
Flavonols		
kaempferol,quercetin, myricetin	+	+
galactose, arabinose, rhamnose	+	+
xylose (in biosides)	rare	-
glucuronic acid	+	rare
5-methylation	-	+
3,5-dimethylation	-	+
8-hydroxylation	-	+
5-glycosylation	rare	-
leucoanthocyanidins	+	+
dihydroflavones	+	+

Table 30. General comparison of flavonoid constituents in the Epacridaceae and the Ericaceae.

D. NUMERICAL ANALYSES

The results of several numerical analyses using data obtained from the chemical survey is given in this section, along with a discussion of their relevance to the classification of the Epacridaceae. No attempt has been made to discuss the philosophy behind numerical taxonomy since this is not the purpose of the thesis. (For a comprehensive treatment of numerical taxonomy and its principles, see Sneath and Sokal - 1973).

A range of programmes involving several different approaches has been used in this work. These include 4 classificatory programmes, MULTBET, CLASS, DIVINF and REMUL. MULTBET was followed in all cases by the diagnostic programme GROUPER, and in specific instances by an ordination programme, GOWER. This latter programme was also used to follow CLASS for the analysis of *Epacris*.

The characteristics and problems associated with these programmes are discussed in Appendix B. A list of definitions of the more common terms used below is given on p. B12 (Appendix B). In this work, the terms "character" and "attribute" are used interchangeably.

1. Treatment of Data

Analyses carried out for data from the Epacridaceae are shown in Table 31. The data used in these was adapted from Table A5 (p. A22) and where applicable, from Table A4 (p. A16). For qualitative analyses, compounds were scored as 0, if absent, and 1, if they occurred in concentrations equal to, or greater than '+' in these tables. For quantitative analyses (run 3), absence was scored as 1, and presence by 2 (= +), 3 (= o), 4 (= ø) and 5 (= •). For most analyses, qualitative data was used even though this represented a serious loss of information, particularly as relationships between species can often be observed through a gradation in character states. However, this limitation was considered preferable once the inaccuracy resulting from the treatment of quantitative characters in MULTBET became apparent (see p. B11).

One computer run (no. 7) was carried out in which the very minor compounds were excluded. The data for this was obtained by removing all compounds scored as '+' in Table A5. Almost certainly, some of these compounds had been misidentified and it was felt that this inaccuracy might influence the classification. (In qualitative data, these minor compounds are weighted equally with very dominant compounds.) In effect, however, this treatment proved worse than leaving the minor

Taxon	Tissue	Compounds	
Family (116 spp.)	L	'flavonoids' (32)	1. MULTBET - GROUPER - GOWER (qualitative)
Family (85 spp.)	Fl	'flavonoids' (19)	2. MULTBET - GROUPER (qualitqtive) 3. MULTBET - GROUPER (ordered multi-state) 4. CLASS (S_J and ISS) 5. CLASS (S_J and Group Average) 6. DIVINF ('normal' and 'inverse')
		minor 'flavonoids' absent (19)	7. CLASS (S_J and ISS)
Family (Tas.) (70 spp.)	L+Fl+Fr+Bk	'flavonoids' and anthocyanins (41)	8. MULTBET - GROUPER (qualitative) 9. CLASS (S_J and ISS)
Styphelieae (33 spp.)	L+Fl+Fr	'flavonoids' and anthocyanins (72)	10. MULTBET - GROUPER (qualitative) 11. CLASS (S_J and ISS)
Cyathodes (11 spp.)	L+Fl+Fr	'flavonoids' and anthocyanins (56)	12. MULTBET - GROUPER (qualitative) 13. CLASS (S_J and ISS)
Epacris (18 spp.)	L+Fl L+Fl	'flavonoids' 'flavonoids'	14. MULTBET - GROUPER - GOWER (qualitative) 15. CLASS (S_J and ISS) - GOWER 16. CLASS (S_K and ISS) - GOWER 17. DIVINF ('normal' and 'inverse') 18. REMUL

Table 31. Computer analyses undertaken with chemical data from the Epacridaceae.

constituents unaltered since it removed more correct identifications than probable misidentifications. Consequently, the procedure was not adopted as routine. However, it was decided to exclude Qu-3-Rha and Km-3-Gal from the following runs (8 - 18) because the incidence of misidentification between these two was undoubtedly high, even at the more significant levels of concentration (see p. 52).

In the initial computer runs (1 - 7), where the number of species was large, 'flavonoids' were used since these represented the largest block of comparable data. In later runs (8 - 13), anthocyanin data were also included. These were not used for flowers and leaves of *Epacris* (runs 14 - 18) because species patterns showed little variation with respect to anthocyanins, or were irregularly contaminated by bark pigments.

Apart from runs 8 and 9, pigments from different tissues were treated as separate attributes. In runs 8 and 9, species were scored for the presence of a particular compound irrespective of the number or type of tissue it occurred in.

In runs 12 - 18, only Tasmanian species, were considered even though data were available for some mainland species. This procedure was adopted because only a superficial knowledge of species from outside Tasmania had been gained and this was not considered adequate to discriminate between 'true' taxonomic relationships and those arising as a result of the procedures employed.

Species variation has been taken into account only in terms of a single representative chromatogram which encompassed all the known chemical variation (see p. 58). For practical reasons, individual chemical variants have not been treated separately.

2. Results and Discussion

Initially, leaf data for all available species were processed using the classificatory programme MULTBET since programmes using the information statistic are recommended for binary data (Williams *et al.* - 1970), and in the light of no prior experience of these procedures it seemed a fair starting point. However, the computing time for such a large number of species was so high, and the resulting classification so poor that it was decided to proceed with smaller numbers and a different tissue type. To this end, a variety of computer programmes were chosen using the 85 species for which flower data were available. Again, however, the results were unsatisfactory and the exclusion of minor constituents resulted in no obvious improvement. Furthermore, the

relative merits of the different classificatory procedures were difficult to ascertain because the results were so confused i.e. species from different genera and subfamilies in the Epacridaceae formed mixed groups in the dendrograms. To illustrate these results, the classificatory groups from runs 4 and 11 (CLASS) are shown in Table 32 (p.103). For run 4, the groups shown have been obtained by terminating the classification at the 20-group level, and for run 11, at the 12-group level. The latter run has been included to represent classificatory attempts at the generic level. Along with run 10 (not shown), it shows some slight improvement over the earlier runs i.e. a higher proportion of species thought to be related from morphological evidence are placed together. Nevertheless, most groups contain species from several genera. To a limited extent, this may reflect a tendency to form ecological groups e.g. *Cyathodes dealbata* and *Monotoca empetrifolia* are high altitude species whilst *Leucopogon parviflorus, L.ericoides, L.collinus, Astroloma humifusum* and *Styphelia adscendens* are usually present in lowland sandy heaths. At the same time, there are species in the classificatory groups which appear unconnected ecologically or morphologically e.g. *Trochocarpa gunnii - Cyathodes* var. *pendulosa* and *Pentachondra pumila - Cyathodes abietina,* etc. As a result of runs 10 and 11, it was not considered worthwhile to continue with the subfamily Epacrideae.

From the results obtained here, it is clear that flavonoid compounds, considered as they were in these numerical analyses, do not contribute significantly towards a meaningful classification of the family.

Two genera, *Epacris* and *Cyathodes*, were selected to test the data at the species level. The results seemed promising and several comparative runs were undertaken with *Epacris*. This genus is large enough to observe meaningful relationships among the species and small enough to avoid confusion from a mass of data. A comparison between the numerical methods attempted unsuccessfully in the earlier runs was repeated in this genus. Other genera were not considered in as much detail as *Epacris* either because of the smaller number of species present or because of the smaller number for which comparable data were available.

(a) Species relationships in *Epacris* and *Cyathodes*

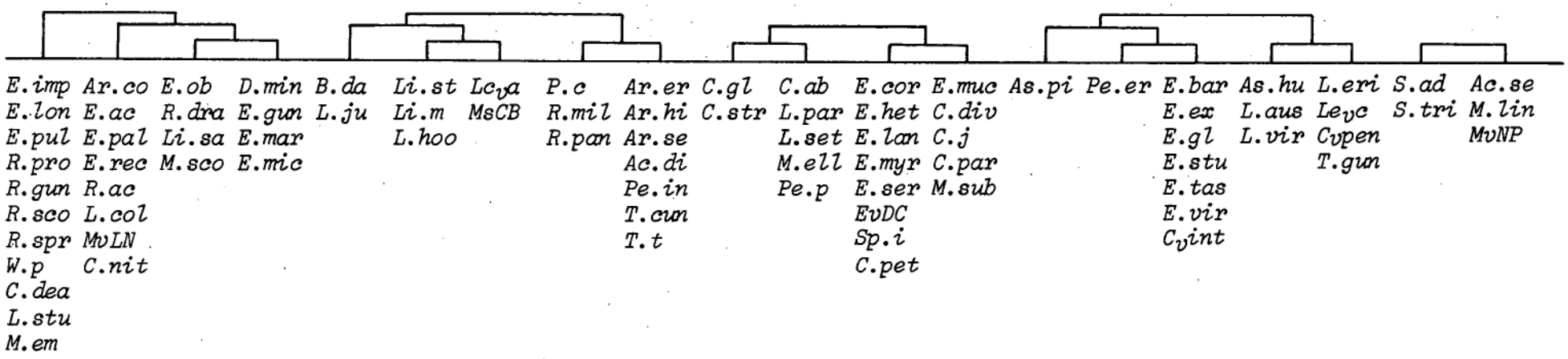
EPACRIS

The classification for *Epacris* obtained from MULTBET and CLASS are shown in Fig. 12. The classification from REMUL, and the 'normal' and

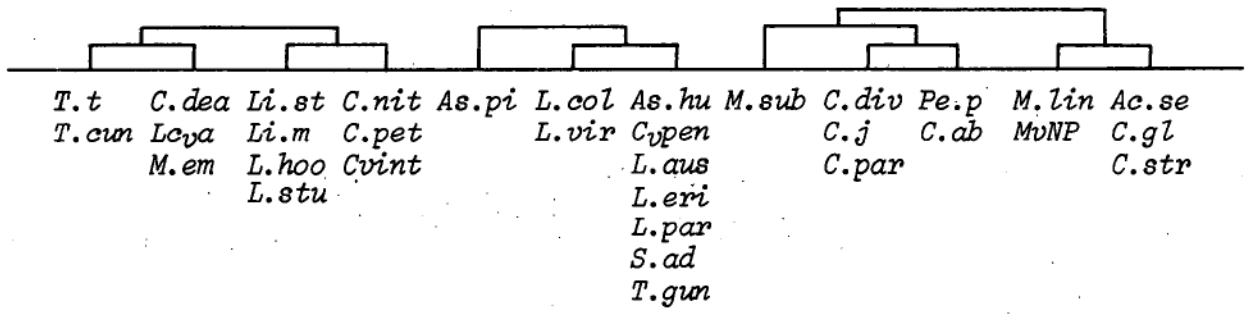
Table 32. Classificatory groups obtained from Runs 4 and 11 (CLASS-S_J - The Epacridaceae & The Styphelieae).

Abbreviations:

Ar.co	-	*Archeria comberi*
Ar.er		*A.eriocarpa*
Ar.hi		*A.hirtella*
Ar.se		*A.serpyllifolia*
Ac.di		*Acrotriche divaricata*
Ac.se		*A.serrulata*
As.hu		*Astroloma humifusum*
As.pi		*A.pinifolium*
B.da		*Brachyloma daphnoides*
C.ab		*Cyathodes abietina*
C.dea		*C.dealbata*
C.div		*C.divaricata*
C.gl		*C.glauca*
C.j		*C.juniperina*
C.nit		*C.nitida*
C.par		*C.parvifolia*
C.pet		*C.petiolaris*
C.str		*C.straminea*
Cvint		*C.*var. *intermedia*
Cvpen		*C.*var. *pendulosa*
D.min		*Dracophyllum minimum*
E.ac		*Epacris acuminata*
E.bar		*E.barbata*
E.cor		*E.corymbiflora*
E.ex		*E.exerta*
E.gla		*E.glabella*
E.gun		*E.gunnii*
E.het		*E.heteronema*
E.imp		*E.impressa*
E.lan	-	*Epacris lanuginosa*
E.lon		*E.longiflora*
E.mar		*E.marginata*
E.mic		*E.microphylla*
E.myr		*E.myrtifolia*
E.muc		*E.mucronulata*
E.ob		*E.obtusifolia*
E.pal		*E.paludosa*
E.pul		*E.pulchella*
E.rec		*E.reclinata*
E.ser		*E.serpyllifolia*
E.stu		*E.stuartii*
E.tas		*E.tasmanica*
E.vir		*E.virgata*
EvDC		*E.*var.*Davies Ck.*
L.aus		*Leucopogon australis*
L.col		*L.collinus*
Lcva		*L.collinus* var. *alpina*
L.eri		*L.ericoides*
Levc		*L.ericoides* var. *coastal*
L.hoo		*L.hookeri*
L.ju		*L.juniperinus*
L.par		*L.parviflorus*
L.set		*L.setiger*
L.stu		*L.stuartii*
L.vir		*L.virgatus*
Li.m		*Lissanthe montana*
Li.sa		*L.sapida*
Li.st		*L.strigosa*
M.ell	-	*Monotoca elliptica*
M.em		*M.empetrifolia*
M.lin		*M.linifolia*
M.sco		*M.scoparia*
M.sub		*M.submutica*
MsCB		*M.submutica* (from Coles Bay)
MvLN		*M.*var.*L.Nicholls*
MvNP		*M.*var.*National Park*
P.c		*Prionotes cerinthoides*
Pe.er		*Pentachondra ericaefolia*
Pe.in		*P.involucrata*
Pe.p		*P.pumila*
R.ac		*Richea acerosa*
R.dra		*R.dracophylla*
R.gun		*R.gunnii*
R.mil		*R.milliganii*
R.pan		*R.pandanifolia*
R.pro		*R.procera*
R.sco		*R.scoparia*
R.spr		*R.sprengelioides*
S.ad		*Styphelia adscendens*
S.tri		*S.triflora*
Sp.i		*Sprengelia incarnata*
T.cun		*Trochocarpa cunninghamii*
T.gun		*T.gunnii*
T.t		*T.thymifolia*
W.p		*Woollsia pungnes*



Run 4. The classification from CLASS (S_J) terminated at the 20-group level. The Epacridaceae



Run 11. The classification from CLASS (S_J) terminated at the 12-group level.
The Tasmanian Styphelieae.

'inverse' classification from DIVINF are shown in Fig. 13.

Ordination graphs showing the first two principal co-ordinates are shown in Fig. 16 (p. 113) for MULTBET, CLASS (S_J) and CLASS (S_K).

(i) *E.gunnii, E.impressa, E.obtusifolia*

These three species form a small group which is clearly separated from the remaining species. In all cases, *E.gunnii* and *E.impressa* appear closer to each other than to *E.obtusifolia*. Using morphological data (see p. 118), these three species are placed among the 'miscellaneous' species outside the two main groups in the genus.

(ii) *E.serpyllifolia, E.corymbiflora, E.heteronema, E.lanuginosa*

These four species consistently form a group in which *E.serpyllifolia* is probably the central species. *E.lanuginosa* is placed at the edge of the group. The close association between these four species supports the relationship indicated by the morphological characters (see p. 118).

(iii) *E.exerta, E.glabella, E.virgata-E.stuartii*

The original data for *E.virgata* and *E.stuartii* was the same and consequently, the two are treated as a single species in these analyses.

The group formed by these species is not as distinct as the preceding two groups but nevertheless, the four species remain associated in all treatments.

(iv) *E.acuminata*

This species is consistently placed with *E.virgata-E.stuartii, E.glabella* and *E.exerta* in all classifications but an examination of the principal co-ordinate analysis indicates that it is apparent at the extreme edge of the group. In CLASS (S_J), *E.acuminata* is further from group (iii) above, than is *E.tasmanica-E.barbata*.

(v) *E.tasmanica-E.barbata*

These two species are treated identically, again because the original data was the same for the two. They occupy an intermediate position between groups (ii) and (iii) above.

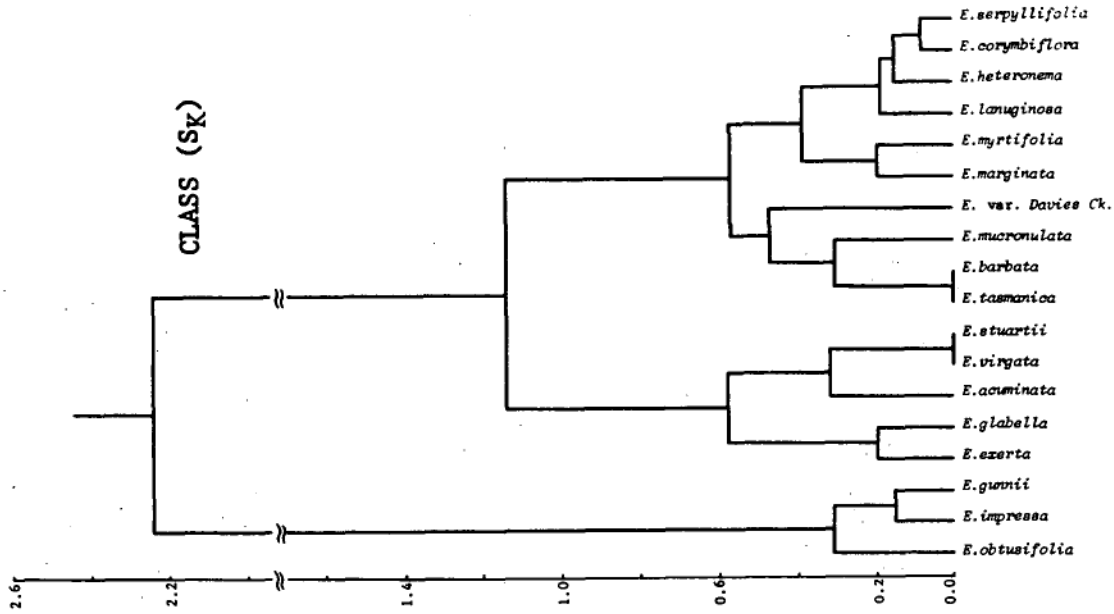
Morphologically, these two species are placed with species from group (iii) but because of their accumulation of myricetin derivatives, they have remained on the outside of this group in all the numerical analyses (see p. 113).

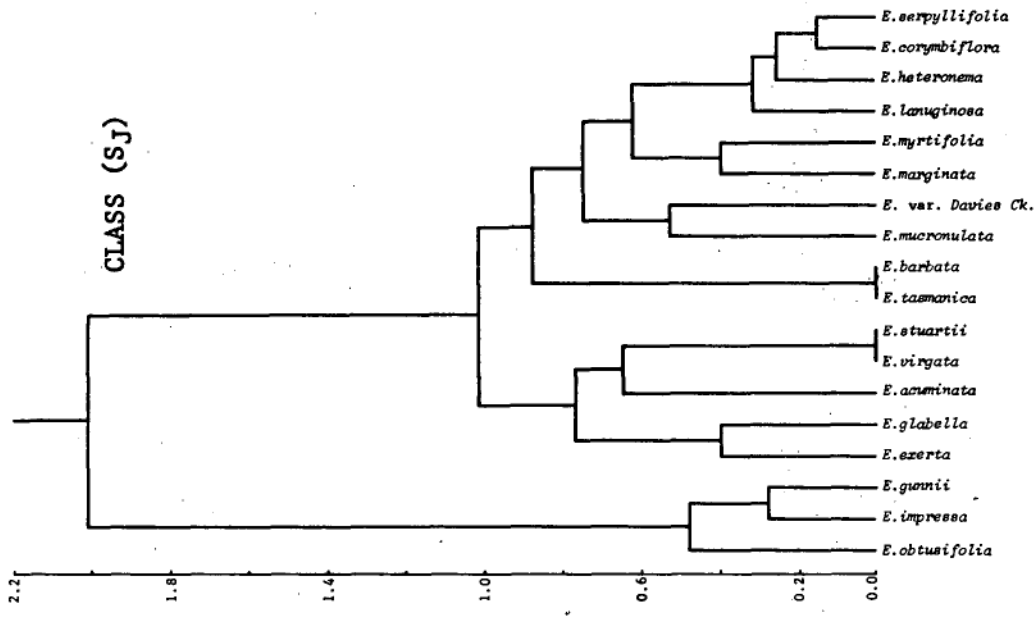
(vi) *E.marginata, E.myrtifolia*

These two species are consistently placed together in the dendrograms but their position with respect to other species varies.

Fig. 12. Classifications obtained for *Epacris* using MULTBET, CLASS (S_J) and CLASS (S_K).

(Vertical axes represent information gain.)


CLASS (S_K)
E.serpyllifolia
E.corymbiflora
E.heteronema
E.lanuginosa
E.myrtifolia
E.marginata
E. var. Davies Ck.
E.mucronulata
E.barbata
E.tasmanica
E.stuartii
E.virgata
E.acuminata
E.glabella
E.exerta
E.gunnii
E.impressa
E.obtusifolia
2.6
2.2
1.4
1.0
0.6
0.2
0.0


CLASS (S_J)
E.serpyllifolia
E.corymbiflora
E.heteronema
E.lanuginosa
E.myrtifolia
E.marginata
E. var. Davies Ck.
E.mucronulata
E.barbata
E.tasmanica
E.stuartii
E.virgata
E.acuminata
E.glabella
E.exerta
E.gunnii
E.impressa
E.obtusifolia
2.2
1.8
1.4
1.0
0.6
0.2
0.0

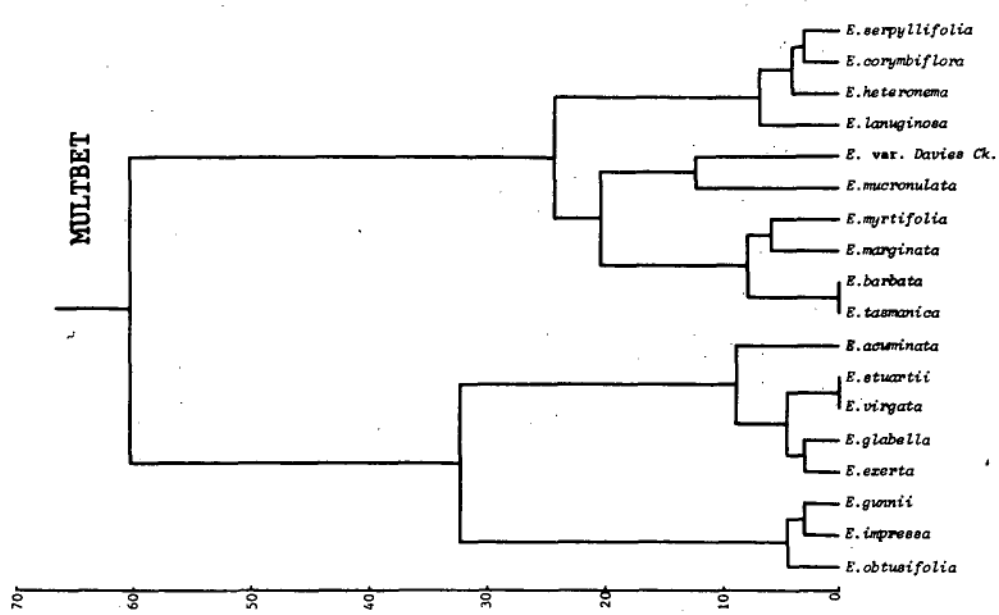
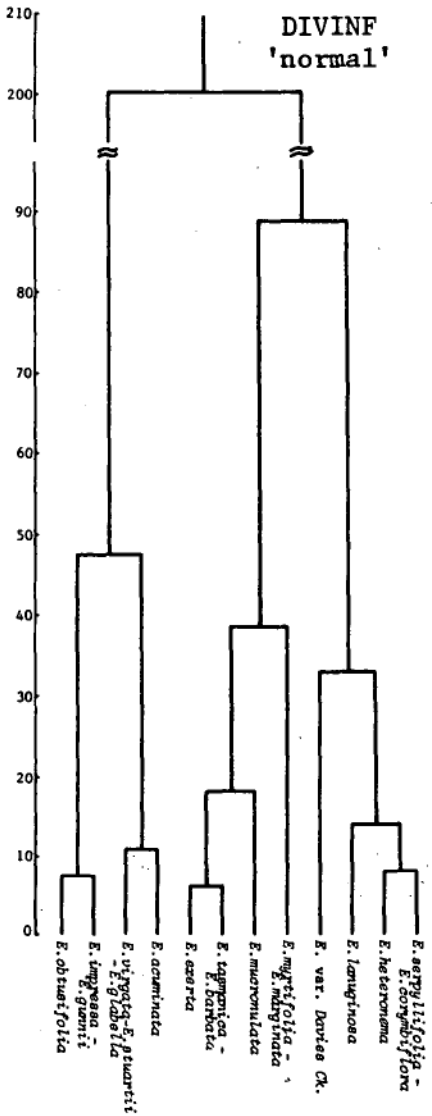
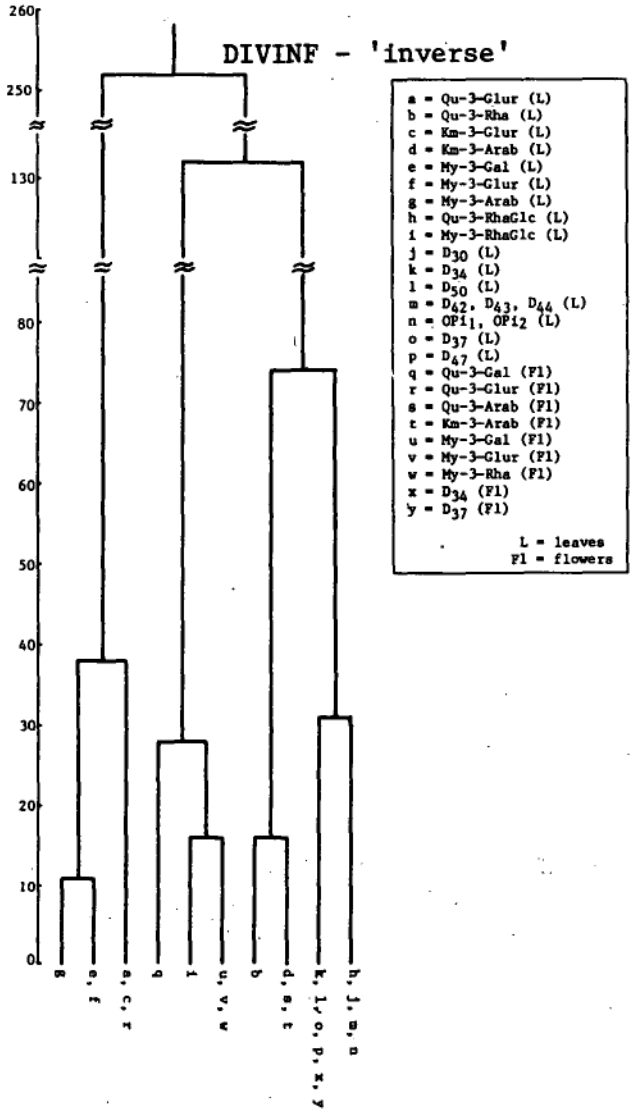
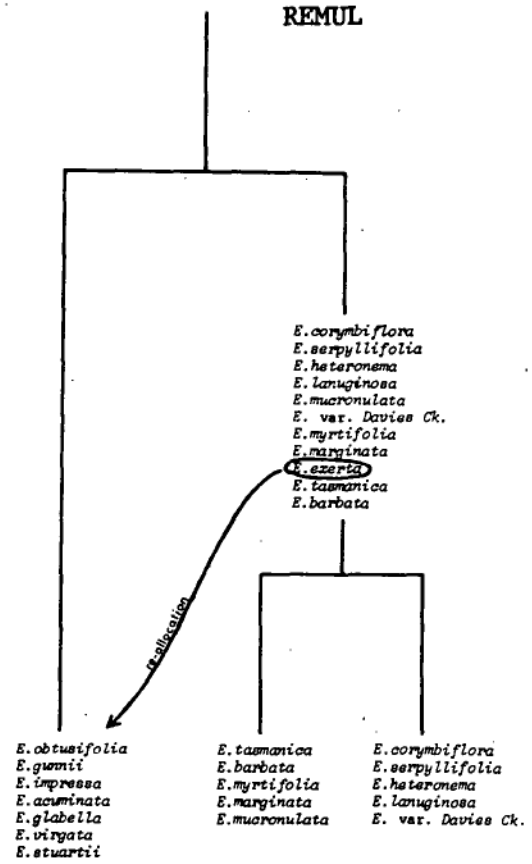

MULTBET
E.serpyllifolia
E.corymbiflora
E.heteronema
E.lanuginosa
E. var. Davies Ck.
E.mucronulata
E.myrtifolia
E.marginata
E.barbata
E.tasmanica
E.acuminata
E.stuartii
E.virgata
E.glabella
E.exerta
E.gunnii
E.impressa
E.obtusifolia
70
60
50
40
30
20
10
0

Fig. 13. Classifications obtained from *Epacris* using DIVINF ('normal' and 'inverse') and REMUL.

(Vertical axes represent information fall.)


DIVINF
'normal'
210
200
90
80
70
60
50
40
30
20
10
0
E.obtusifolia
E.gunnii - E.glaucina
E.impressa - E.stuartii - E.glabella
E.virgata
E.acuminata
E.exserta
E.tasmanica - E.barbata
E.mucronulata
E.myrtifolia - E.marginata
E. var. Davies Ck.
E.lanuginosa
E.heteronema
E.serpyllifolia - E.corymbiflora


DIVINF - 'inverse'
260
250
130
80
70
60
50
40
30
20
10
0
a = Qu-3-Glur (L)
b = Qu-3-Rha (L)
c = Km-3-Glur (L)
d = Km-3-Arab (L)
e = My-3-Gal (L)
f = My-3-Glur (L)
g = My-3-Arab (L)
h = Qu-3-RhaGlc (L)
i = My-3-RhaGlc (L)
j = D30 (L)
k = D34 (L)
l = D50 (L)
m = D42, D43, D44 (L)
n = OPi1, OPi2 (L)
o = D37 (L)
p = D47 (L)
q = Qu-3-Gal (Fl)
r = Qu-3-Glur (Fl)
s = Qu-3-Arab (Fl)
t = Km-3-Arab (Fl)
u = My-3-Gal (Fl)
v = My-3-Glur (Fl)
w = My-3-Rha (Fl)
x = D34 (Fl)
y = D37 (Fl)
L = leaves
Fl = flowers
g
e, f
a, c, r
b
i
u, v, w
d, s, t
k, l, o, p, x, y
h, j, m, n


REMUL
E.corymbiflora
E.serpyllifolia
E.heteronema
E.lanuginosa
E.mucronulata
E. var. Davies Ck.
E.myrtifolia
E.marginata
E.exserta
E.tasmanica
E.barbata
re-allocation
E.obtusifolia
E.gunnii
E.impressa
E.acuminata
E.glabella
E.virgata
E.stuartii
E.tasmanica
E.barbata
E.myrtifolia
E.marginata
E.mucronulata
E.corymbiflora
E.serpyllifolia
E.heteronema
E.lanuginosa
E. var. Davies Ck.

From the principal co-ordinate graphs, they appear intermediate between groups (ii) and (v) above.

It seems likely that these two species are forming an ecological group and their chemical similarity results from a similar response to a harsh environment (exposed, windswept sea coast). Morphologically they do not appear to be closely related.

(vii) *E.mucronulata, E.* var. *Davies Ck.*

Excepting for the classification from DIVINF, these two species tend to be associated in all the dendrograms. However, from the ordination graphs, it is apparent that they are well separated, with *E.mucronulata* appearing closest to *E.tasmanica-E.barbata* whilst *E.* var. *Davies Ck.* is closest to *E.heteronema.* From morphological data, *E.* var. *Davies Ck.* is considered intermediate between *E.mucronulata* and *E.serpyllifolia* (see p. 132).

CYATHODES

The two classifications for *Cyathodes* are shown in Fig. 14 (p. 108). A principal co-ordinate analysis was not undertaken, but in retrospect, this would obviously have been worthwhile.

Most species in the genus are treated in a comparable manner by the two programmes, and the groups formed agree in general with morphological data. The three groups were -

(i) *C.juniperina, C.parvifolia, C.divaricata*

(ii) *C.glauca, C.straminea*

(iii) *C.petiolaris, C.nitida, C.* var. *intermedia, C.abietina, C.dealbata.*

Although *C.dealbata* is placed in group (iii), it occurs at the edge of the group. Neither its occurrence in this group, nor that of *C.abietina* is strongly supported by morphological evidence, and the fusion of group (ii) with (i) rather than (iii) is unexpected (see Fig. 14).

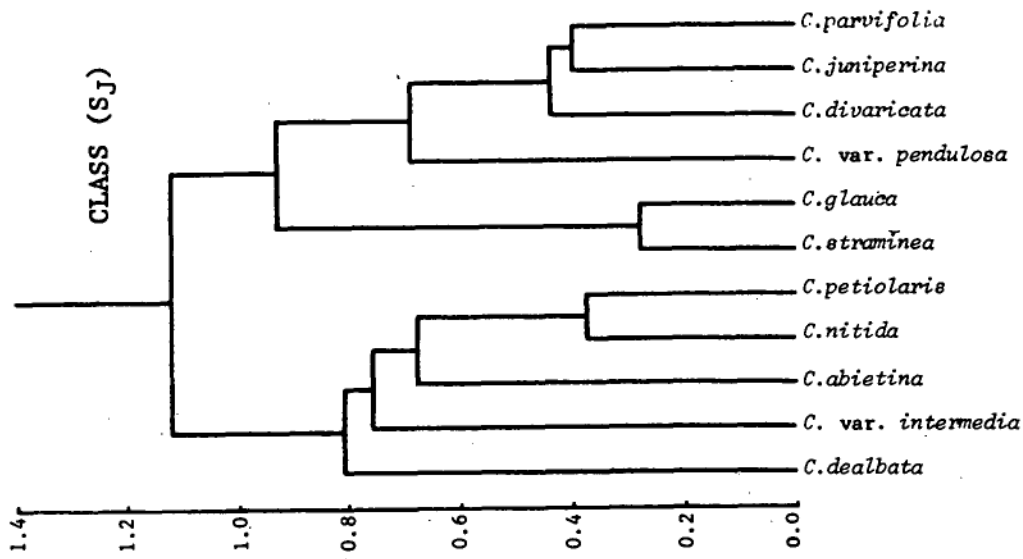
The position of *C.* var. *pendulosa* varies between the first group in CLASS and the third group in MULTBET. The former arrangement is indicated by morphological data.

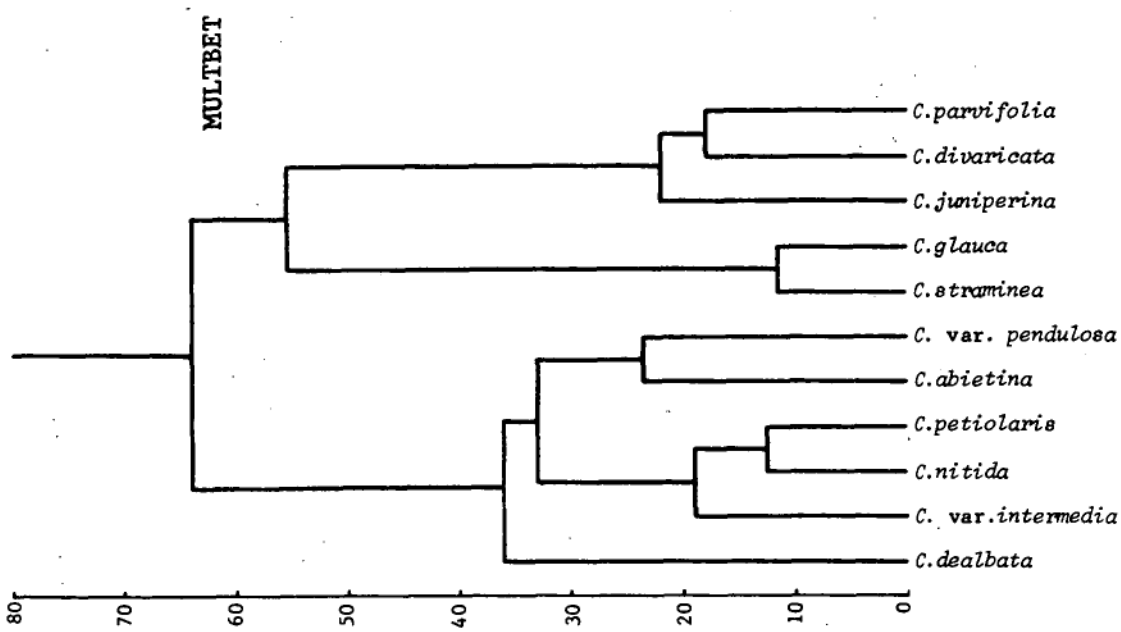
(b) Chemical characters

From an examination of GOWER (MULTBET), DIVINF and REMUL, it is possible to obtain the identity of compounds involved in fusions and divisions of the respective classifications. The same compounds are

Fig. 14. Classifications obtained for *Cyathodes* using MULTBET and CLASS (S_J).

(Vertical axes represent information gain.)


CLASS (SJ)
C.parvifolia
C.juniperina
C.divaricata
C. var. pendulosa
C.glauca
C.straminea
C.petiolaris
C.nitida
C.abietina
C. var. intermedia
C.dealbata
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0


MULTBET
C.parvifolia
C.divaricata
C.juniperina
C.glauca
C.straminea
C. var. pendulosa
C.abietina
C.petiolaris
C.nitida
C. var.intermedia
C.dealbata
80
70
60
50
40
30
20
10
0

apparently used in DIVINF and REMUL, and only the former classification will be considered here.

In Fig. 15 (p. 110), the two classificatory programmes MULTBET and DIVINF are shown terminated at the 5-group level. Compounds involved at the various levels of fusion (obtained from GROUPER) and division are shown. The arrow placed beneath the compounds at each node indicates which of the two groups is responsible for information contributed about these compounds. In MULTBET, fusions are based on the occurrence of several compounds none of which is necessarily mutually exclusive in the two groups fusing. In the classification shown, the compounds are listed at each node in the order of importance, with the pigment responsible for the highest information contribution at the top of the list. In DIVINF, divisions are based on a single character which is mutually exclusive in the two groups.

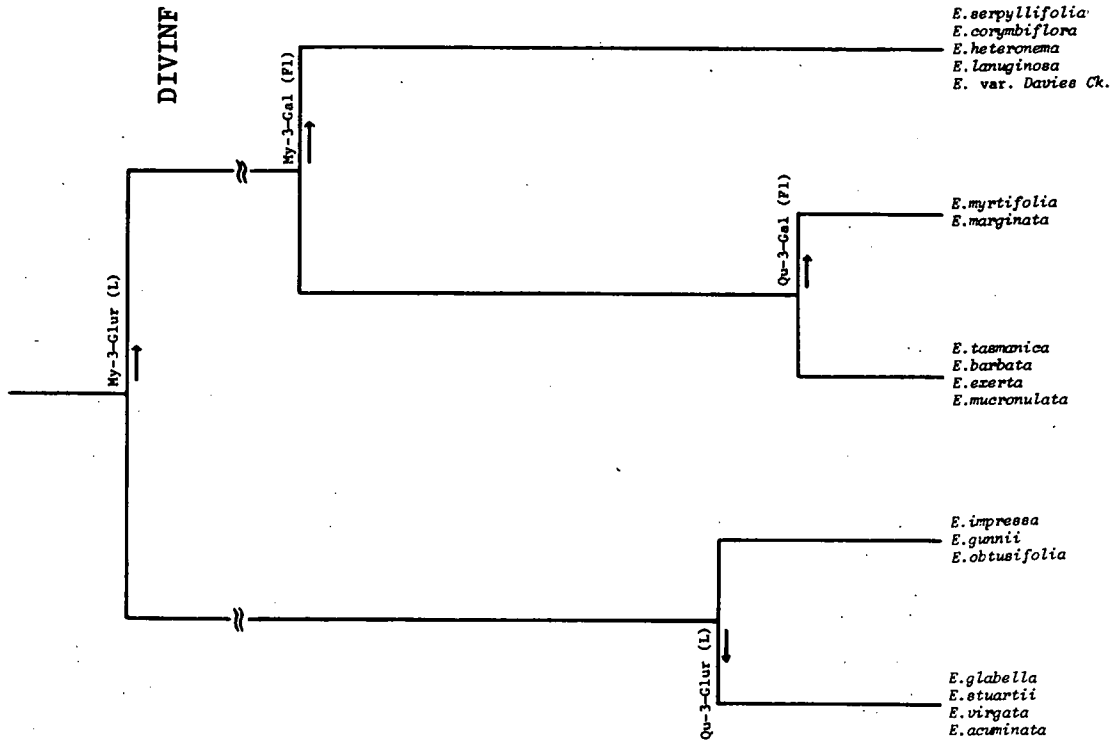
From Fig. 15, it is apparent that the aglycone myricetin, and the sugar, glucuronic acid, offer the most significant contribution to the classifications.

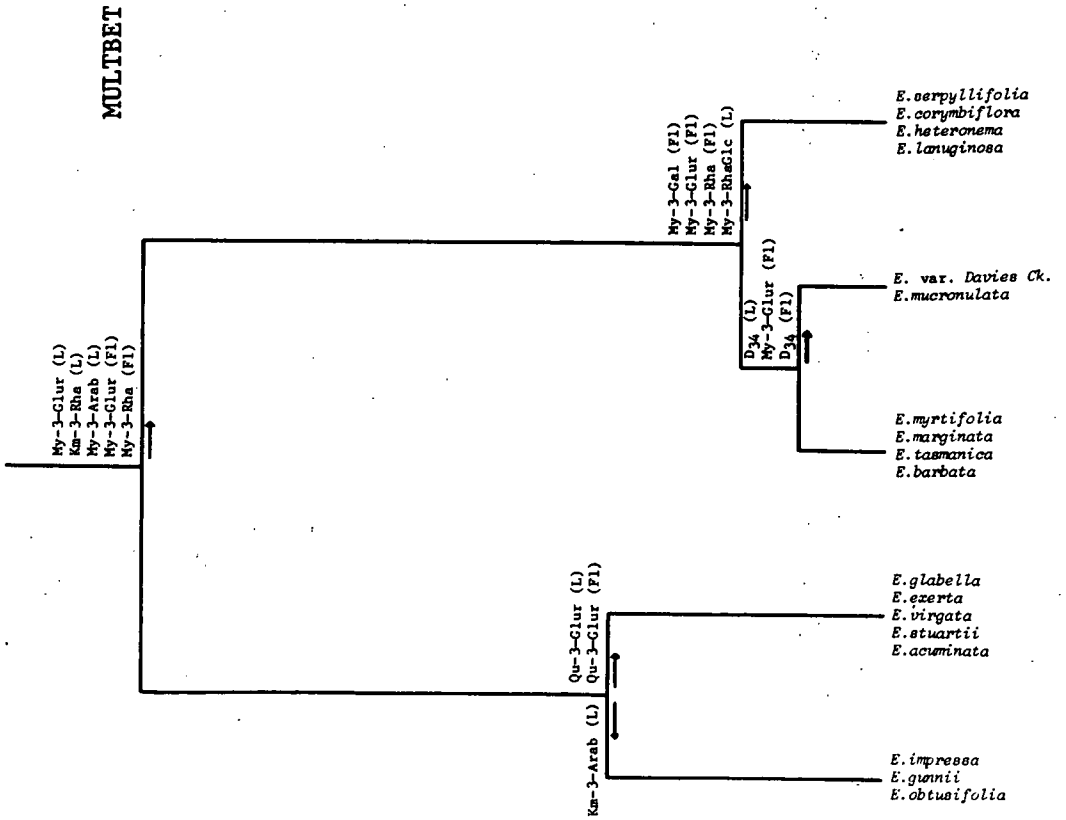
From the 'normal' and 'inverse' classifications obtained from DIVINF, it is possible to construct a contingency table showing groups of species characterized by groups of compounds. Initially, a 4 × 6 contingency table was constructed, in which the 'normal' classification was terminated at the 4-group level and 'inverse' classification was terminated at the 6-group level (see Fig. 13). However, trends in the table were obscured because of species-specific compounds and compounds of sporadic occurrence. Using this table as a guide, a 4 × 4 table was constructed in which the rare compounds were eliminated (see Table 33, p. 111). Compounds such as Qu-3-Gal (flowers) and My-3-Arab (leaves) have also been excluded since their distribution tends to obscure the patterns otherwise apparent. Values shown in the table are obtained by adding the number of times each pigment occurs in each group and dividing by the sum of the total possible occurrences of each pigment in the group.

From Table 33, it is apparent that in the Epacridaceae, compounds with the same sugar derivatives tend to be associated irrespective of the aglycone, and this association is consistent between the different tissues.

The potential of this procedure in determining the distribution patterns of compounds is clear from this work, particularly for large numbers of species and characters.

Fig. 15. Compounds involved in major fusions or divisions in MULTBET and DIVINF.


DIVINF
My-3-Glur (L)
My-3-Gal (F1)
Qu-3-Gal (F1)
Qu-3-Glur (L)
E.serpyllifolia
E.corymbiflora
E.heteronema
E.lanuginosa
E. var. Davies Ck.
E.myrtifolia
E.marginata
E.tasmanica
E.barbata
E.exerta
E.mucronulata
E.impressa
E.gunnii
E.obtusifolia
E.glabella
E.stuartii
E.virgata
E.acuminata


MULTBET
My-3-Glur (L)
Km-3-Rha (L)
My-3-Arab (L)
My-3-Glur (F1)
My-3-Rha (F1)
My-3-Gal (F1)
My-3-Glur (F1)
My-3-Rha (F1)
My-3-RhaClc (L)
D34 (L)
My-3-Glur (F1)
D34 (F1)
Qu-3-Glur (L)
Qu-3-Glur (F1)
Km-3-Arab (L)
E.serpyllifolia
E.corymbiflora
E.heteronema
E.lanuginosa
E. var. Davies Ck.
E.mucronulata
E.myrtifolia
E.marginata
E.tasmanica
E.barbata
E.glabella
E.exerta
E.virgata
E.stuartii
E.acuminata
E.impressa
E.gunnii
E.obtusifolia

Species groups	Qu3Glur (L) Qu3Glur (F1) Km3Glur (L)	My3Gal (L) My3Glur (L)	Km3Arab (L) Qu3Arab (F1) Km3Arab (F1)	My3Gal (F1) My3Glur (F1)
E.barbata *E.tasmanica* *E.exerta* *E.marginata* *E.mucronulata* *E.myrtifolia*	16/18	11/12	1/18	1/12
E.corymbiflora *E.heteronema* *E.lanuginosa* *E.serpyllifolia* *E.* var. *Davies Ck.*	15/15	10/10	0/15	10/10
E.acuminata *E.glabella* *E.stuartii* *E.virgata*	11/12	0/8	2/12	0/12
E.gunnii *E.impressa* *E.obtusifolia*	0/9	2/6	7/9	0/6

Table 33. A 4 × 4 contingency table constructed from the 'normal' and 'inverse' classifications of DIVINF.

(c) Comparison of procedures

Various aspects of the procedures used here are discussed in detail in Appendix B (pp. B8 - B11). Although much of the discussion is pertinent to this section, it was felt that because of its general nature, it would be better placed near the programme descriptions. More specific aspects applicable to the Epacridaceae are dealt with here. Although the discussion is orientated towards *Epacris*, the principles involved also refer to analyses carried out elsewhere in the family.

(i) Classificatory programmes

The classifications obtained from runs 14 - 18 are shown in Figs. 12 and 13 (p. 105 and p. 106, respectively). In these classifications, the order of fusion and division has been considered but the information levels, in terms of absolute values, have been ignored.

Differences between the classifications are minor and consequently none stands out as being distinctly better or worse than the alternatives. From a comparison of REMUL and DIVINF, it was possible to detect one misclassified species (*E.exerta*) in the results from the latter programme. However, misclassified species may also be present in the other

classifications but without a similar re-allocation procedure (see Appendix B), they have remained unnoticed.

In terms of taxonomic significance, the results of CLASS (S_J) most closely approach the relationships suggested by morphological data (see Part III, p. 118). However, on the basis of the argument used below (p. 114), it cannot be claimed that this procedure is superior to the alternatives.

(ii) Principal co-ordinate analysis

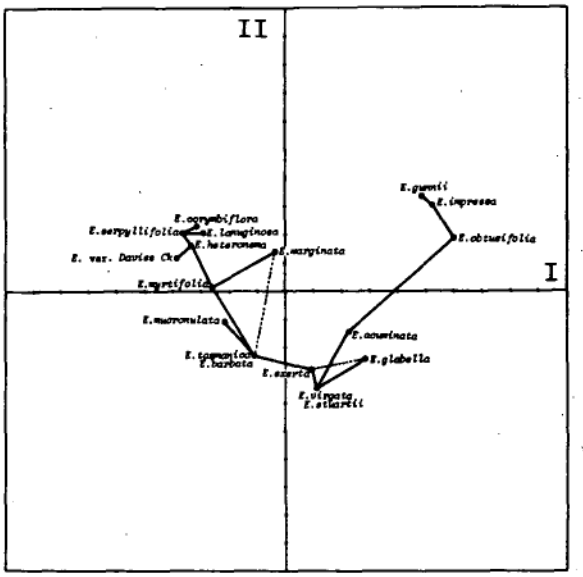
Ordination of the principal co-ordinates (eigenvectors) obtained from GOWER for MULTBET and CLASS (S_J and S_K) are shown in Fig. 16 (p. 113). In all cases, the total variance encompassed by the first three eigenvectors is greater than 50% i.e. CLASS (S_J) = 53.8%, CLASS (S_K) = 66.6% and MULTBET = 63.6%. The greater part of this variance is included in the first two vectors (i.e. 43.8%, 57.1% and 52.1%, respectively) and for this reason, only these have been plotted in Fig. 16. On each graph, the minimum spanning tree (see p. B7) has been superimposed over the ordination plots, with the distances between points being obtained from the original dissimilarity matrices (p. B13). Where species are equidistant from the nearest neighbour, this is demonstrated by a dotted line.

From an inspection of these graphs, it is apparent that some distortion has arisen from the visual representation of species (points) in a two dimensional field. For example, *E.corymbiflora* and *E.lanuginosa* appear spatially close but in fact, each is nearer to *E.serpyllifolia*, even though this latter species appears more distant on the graph. However, this distortion does not interfere significantly with the recognition of groups among the species, providing the minimum spanning tree is always used.

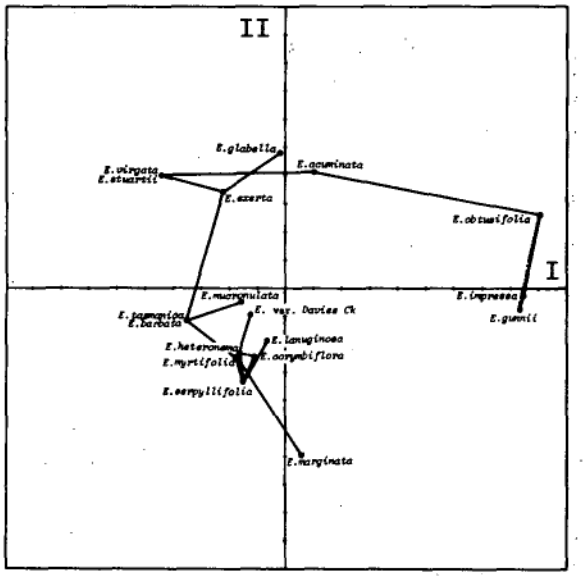
The principal co-ordinate analysis is particularly useful for visualizing the relationships within a group. Although this is apparent to some extent from the classifications, there is free rotation at each node in the dendrogram so that the situation between adjacent groups or individuals is not clear. A further advantage with ordination analyses includes the ability to demonstrate the arrangement of species within the sub-groups i.e. it shows if a central species exists to which all others are closely related, or if the species are related in a linear fashion. It is not possible to determine this from the dendrograms.

Some discrepancies may be apparent from relationships suggested

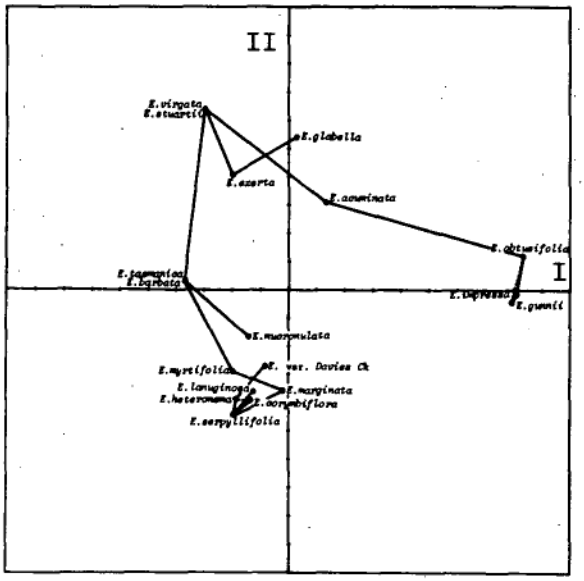
Fig. 16. Ordination graphs showing the first two principal co-ordinates obtained from MULTBET, CLASS (S_J) and CLASS (S_K).



MULTBET



CLASS (S_J)



CLASS (S_K)

from the dendrograms and those seen in the ordination analyses. For example, *E.tasmanica-E.barbata* are either equidistant from, or closer to, *E.exerta, E.glabella, E.stuartii-E.virgata* than is *E.acuminata* but this is not apparent in any of the dendrograms.

The difference between relationships suggested from the multivariate analysis and the classificatory programmes results from the use of the original dissimilarity measures in the former and modified dissimilarity measures in the latter i.e. in the dendrograms, a new dissimilarity measure is calculated at each fusion. Although a given species may be closest to one of the fused species, it may not be similar to the particular group as a whole. Consequently, it is not necessarily included in the group even though its closest affinities are with one of the constituent species.

From an examination of these results, it is clear that the construction of a classification is not sufficient in itself to show relationships between species, and the inclusion of a principal co-ordinate analysis is essential.

3. General Summary

In the analyses carried out here, it is virtually impossible to choose the 'best' classificatory programme. An objective assessment is difficult to achieve because there is no established numerical taxonomic standard to which the results may be related. The only comparison available is with a more conventional form of taxonomy i.e. classical type studies in morphology, anatomy,etc. Since work of this latter kind is itself subjective, its use in evaluating the different numerical techniques is dubious.

There is undoubtedly a tendency in this work to consider the best numerical results as those which conform most closely to the particular classification advocated by the taxonomist carrying out the analyses. However, there is such a range of similarity measures, clustering strategies and alternative ways of coding the data, that the potential for manipulating the results is considerable. Hence, any claims that numerical taxonomy is 'objective' must be treated with suspicion. It is objective only in the actual calculations undertaken and the outcome is as susceptible to subjective reasoning as the more conventional form of taxonomy. Nevertheless, if used cautiously and in conjunction with conventional studies, numerical procedures are undoubtedly a valuable

asset to the study of taxonomy, particularly in their capacity to store and process large quantities of data at speed. Furthermore, species relationships hitherto unsuspected amongst the mass of data, may become apparent. Once, detected, the validity of these relationships may then be assessed by other means.

E. CONCLUDING REMARKS

The work presented in this thesis represents only the beginning of research into the family Epacridaceae. Nevertheless, the results indicate the potential of flavonoid compounds in both taxonomic and phylogenetic studies. At the same time, accompanying research in morphology, cytology, etc. is necessary to do justice to these studies. At the lower taxonomic levels, flavonoids are important in identification and in suggesting species and generic relationships. Above the species level, flavonoids alone do not contribute significantly towards the formation of a realistic classification.

Preliminary studies into the procedures of numerical taxonomy demonstrate their capability of handling large quantities of data and reducing it to a manageable size. However, this is a relatively recent field of research and there are still many problems to be resolved.

PART III

OBSERVATIONS ON THREE GENERA IN TASMANIA

In this section, three specific genera i.e. *Epacris*, *Cyathodes* and *Monotoca*, have been examined in some detail and the contribution of flavonoid chemistry in their taxonomy is discussed. The work on these genera is far from complete and for several species, only a very superficial knowledge of their variation has been gained. Whenever appropriate, species descriptions have been given but these are referred to Appendix C. The work is not intended as a revision, although it is hoped that it may form the basis of such a study at some future time.

Tasmanian locations mentioned in the text are given on p. C19.

A. *EPACRIS*

In the most recent treatment of Tasmanian species of *Epacris* (Curtis - 1963), 18 species are recorded with 11 being listed as endemic. The genus is readily distinguished from its two nearest relatives, *Archeria* and *Prionotes*, by the presence of many imbricating bracts on the pedicel. Of the genus as a whole, Bentham (1869) wrote -

> "With all its variations in foliage and shape of the corolla, it is the most easily recognised in the order, differing from all except *Lysinema* in foliage and inflorescence and neatly distinguished from the latter genus by the aestivation of the corolla. The species, however, are exceedingly difficult to circumscribe by any definite characters, the whole eighteen of the short flowered ones seeming to pass into each other by small gradations."

In Tasmania, species range from coastal habitats to alpine situations, from swamps to very dry areas. Some species are widely distributed throughout the state e.g. *E.impressa* and *E.lanuginosa*, but others such as *E.stuartii*, *E.myrtifolia* and *E.marginata* are particularly restricted. Few species exceed 1 m. in height, and those that are taller are usually slender and almost spindly in form. Exceptions include *E.heteronema* which has been reported to form a small tree up to 6 m. on the west coast (Curtis - 1963). In all species, the corolla is

white excepting in *E.impressa* where it may range from white through all shades of pink, to red. The flowers are solitary but often form conspicuous and very attractive displays. All species are hermaphroditic.

1. General relationships among species

On the basis of morphological data, two groups and several 'miscellaneous' species are apparent in Tasmanian representatives of the genus. The major divisions, referred to as E1 and E2 (for convenience), are shown in Table 34 (p. 118). Chemical differences between the two groups are also shown in this table and the actual results from which these were derived are shown in Table 35 (p. 119). (For convenience, this latter table duplicates the results given in Table A5, p. A22, but for clarity the arrangement of species has been altered.) In general, these chemical results support the deductions obtained from the morphological data.

Limited support for the groups is also available from the numerical analyses. (This is to be expected since these analyses are based on the information in Table 35). From numerical work, three groups are apparent in the genus -

(a) *E.exerta, E.glabella, E.virgata, E.stuartii*

(b) *E.corymbiflora, E.serpyllifolia, E.heteronema, E.lanuginosa*

(c) *E.impressa, E.gunnii, E.obtusifolia*

These groups are placed in E1, E2 and 'miscellaneous' species respectively.

Four groups in *Epacris* are given by Hooker (1860) and these markedly cut across the three followed in this work. The constituent species of Hooker's groups are shown in Table 36. His two larger groups are based mainly on differences in the leaf apex - a poor character in this genus where clear cut differences are not always apparent. Chemical evidence obtained during this study does not support Hooker's groups but his divisions may have been intended more for identification purposes rather than to show species similarities.

It is of interest to note that both Hooker (1860) and Bentham (1869) describe *E.obtusifolia* as having a corolla tube scarcely longer than the calyx. In Tasmanian representatives of the species observed during this study, the corolla tube was markedly longer than the calyx.

Group E1

E.tasmanica, E.barbata, E.stuartii, E.exerta, E.glabella, E.virgata

Morphological characters

(i) Anthers exerted beyond the corolla tube.
(ii) Funnel-shaped corolla tube
(iii) Corolla tube approximately equal to the calyx.
(iv) Lobes approximately equal to the tube.
(v) Style exceeding the calyx (after corolla is removed).

Chemical characters

(i) Flavonol glucuronides present in excess of galactosides (the latter are absent from the flowers).
(ii) Km- and Qu-3-Rha dominant in the flowers and greatly in excess of the weak flavonol glucuronides.
(iii) Myricetin derivatives absent from the flowers.

Group E2

E.heteronema, E.mucronulata, E.serpyllifolia, E.corymbiflora, E.lanuginosa, E.paludosa, E. var. *Davies Ck., E.myrtifolia*

Morphological characters

(i) Sessile, or very shortly stalked anthers (not exerted).
(ii) Cylindrical-shaped corolla tube.
(iii) Corolla tube approximately equal to the calyx.
(iv) Style usually shorter than the calyx (after removal of corolla).

Chemical characters

(i) Flavonol glucuronides and galactosides present in approximately equal concentrations.
(ii) Flavonol glucuronides and galactosides regularly produced in the flowers.
(iii) Myricetin derivatives usually present in the flowers.

'Miscellaneous' species

E.acuminata, E.gunnii, E.impressa, E.obtusifolia, E.petrophila

Table 34. Three groups in Tasmanian *Epacris*.

Species	Qu3Gal	Qu3Glur	Qu3Arab	Qu3Rha	Km3Gal	Km3Glur	Km3Arab	Km3Rha	My3Gal	My3Glur	My3Arab	My3Rha	Qu3RhaGlc	My3RhaGlc	D30	D34	D50	D37	D47	D42,43,44	OP11,OP12
Flowers																					
E.tasmanica		+		●				●													
E.barbata		+		●				●													
E.stuartii		+		●				●													
E.exerta		+		●				●													
E.virgata		+		●				●													
E.glabella		+		●				●													
E.serpyllifolia	o	o		●				⊘	+	o		+									
E.heteronema	+	o		●				⊘	+	+		o									
E.mucronulata	o	o	+	●				⊘		+						+					
E. var. Davies Ck.	o	+		●				⊘	o	+						o		o			
E.corymbiflora	o	o		●				+	o	o		⊘									
E.lanuginosa	o	o	+	●				⊘	+	o		+									
*E.paludosa**	o	o	+	●				⊘													
E.myrtifolia	o	o		●				⊘				+				o		o			
E.acuminata	+	o	+	●			⊘	⊘													
E.marginata	+			●				⊘				●									
E.gunnii	o		+	●				●													
E.impressa	●		⊘	o				+													
E.obtusifolia	●		⊘	⊘			o	+													
Leaves																					
E.tasmanica	⊘	●	o		+	+			+	+	+										
E.barbata	⊘	●	o		+	o			o	o	+										
E.stuartii	⊘	●	o		o	o															
E.exerta	⊘	●	+		+	+		+		+											
E.virgata	⊘	●	+		o	o															
E.glabella	⊘	●	+					+													
E.serpyllifolia	●	●	o	+		+			o	o	+			+				o			
E.heteronema	●	●	o	+		+			o	+						+					
E.mucronulata	●	●	o	+		+			⊘	⊘	+				+	+					
E. var. Davies Ck.	●	●	+			+			o	⊘						+	+	o	o		
E.corymbiflora	●	●	o	+		+			+	+			+	+							
E.lanuginosa	●	⊘	o	+		+		+	o	+	+									o	
E.paludosa	●	●	o	+		+		+	+	+			+								
E.myrtifolia	●	●	o	+		+			o	o	+										
E.acuminata	●	⊘	⊘		o	o															
E.marginata	⊘	o	o	+					●	o	o										
E.gunnii	●		⊘	+			+	+	+					+							
E.impressa	●		⊘	+			+	+	o												o
E.obtusifolia	●		⊘	⊘			o	+													
E.petriphila	●		⊘	o				o	o	+	o										

Table 35. Distribution of 'flavonoids' in the genus *Epacris*.

(* *E.paludosa* was not collected in Tasmania but it was included in this comparison because of its close morphological similarity to *E.lanuginosa*.)

Pigment concentration: ● > ⊘ > o > +

E.gunnii
leaves cordate and sheathing at the base
E.impressa, E.ruscifolia, E.ceraeflora (all now included in *E.impressa*)
leaves ovate or lanceolate, tube of corolla more than twice the length of the calyx
E.lanuginosa, E.mucronulata (= E.acuminata Benth.*), E.heteronema, E.squarrosa (= E.tasmanica)*
leaves ovate or lanceolate, not cordate at the base, decidedly pungent, tube of the corolla little, if at all longer than the calyx
E.myrtifolia, E.serpyllifolia, E.exerta, E.virgata, E.obtusifolia, E.franklinii (= E.mucronulata R.Br.*), E.corymbiflora, E.petrophila*
leaves ovate or lanceolate, not cordate at the base, blunt, acute or acuminate, but not pungent, tube of corolla not longer than the calyx

Table 36. Divisions within *Epacris* (taken from Hooker - 1860).

(a) Group E1

On the basis of floral morphology, E1 forms a uniform group which is strongly supported by floral chemistry. Variation is apparent in the leaves but this is consistent with trends found elsewhere in the family (i.e. vegetative variation exceeds floral variation). *E.tasmanica* and *E.barbata* are excluded from group E1 in the numerical analyses because myricetin derivatives are accumulated in the leaves. This is not considered significant however, in the light of intra-specific variation observed in *E.tasmanica*. This species forms a link between *E.barbata* and other species of the group, accumulating myricetin in northern populations but not in southern populations (see p. 126).

(b) Group E2

Species in group E2 are less uniform than those of E1, with respect to both morphological and chemical characters. According to the weighting given to particular characters, the number and identity of species in the group varies. This would suggest perhaps, that the species are too heterogeneous to be placed together at all, and in fact this may be so if only those species at the extremes of morphological variation are considered. However, the fact that these can be linked throughout by intermediate species cannot be overlooked. For example, *E.lanuginosa* and *E.paludosa* are similar to each other in vegetative characters but differ markedly from *E.corymbiflora* and *E.myrtifolia*.

However, *E.mucronulata* and *E.serpyllifolia* are intermediate between these two extremes. In the case of *E.myrtifolia*, floral characters (funnel-shaped corolla tube, half exerted anthers) are not in agreement with the relative uniformity of floral characters shown by other members of the group. However, the general aspect and vegetative parts of *E.myrtifolia* are so similar to those one might expect from a lush form of *E.serpyllifolia*, that one feels intuitively that it should be included in the group. (While intuition is not normally considered a sound basis for scientific reasoning, to some extent it must be defended in taxonomy where it may represent a taxonomist's capacity to integrate minute differences and similarities even while these cannot be easily translated into descriptive terms.) In this particular case, chemical data (see Tables 34 and 35) support the inclusion of *E.myrtifolia* into group E2.

From the numerical analyses, *E.mucronulata* and *E.* var. *Davies Ck.* have not been assigned to group E2 and this may be due to the method of scoring (p. 99). Neither quantitative differences nor intraspecific variation has been taken into account, and the numerical procedures are unable to discriminate between compounds of rare occurrence and those which occur frequently (see p. B8).

(c) 'Miscellaneous' species

Tasmanian species not included in groups E1 and E2 are *E.acuminata*, *E.marginata*, *E.gunnii*, *E.impressa*, *E.obtusifolia* *E.petrophila*. In floral morphology, *E.acuminata* is similar to species of group E1 but the general aspect of the plant and vegetative characters are different (thin concave leaves which are stem clasping at the base). It exclusion from group E1 is supported by the subjective analysis of chemical data, and in particular by the flower flavonols (see Table 35 p. 119). In the numerical analyses, it is placed at the edge of group E1 species, and this would seem reasonable in the light of its morphological characters. Two species, *E.impressa* and *E.obtusifolia*, are perhaps closer to each other morphologically than to the remaining species. In both, the long corolla tube has relatively short lobes and the style exceeds the calyx. Chemical similarities between the two include the accumulation of dominant flavonol galactosides and arabinosides, the absence of flavonol glucuronides and the presence of rhamnosides as minor constituents only. However, the relationship suggested here is not consistent with that indicated in the numerical analyses in which *E.impressa* appears closer to *E.gunnii* than to *E.obtusifolia*.

2. Comments on some species

Before undertaking a discussion of individual species, it is relevant to mention briefly one aspect of plant variation.

Many plant species in Tasmania show modifications in form between inland and coastal habitats. Some morphological adaptions in coastal areas include broader and thicker leaves or phyllodes (e.g. *Epacris impressa, Leucopogon ericoides, Leptospermum scoparium, Acacia verticillata,* etc.), less recurved leaves (e.g. *Epacris impressa*), more crowded leaves (e.g. *Leucopogon ericoides*) and in very exposed areas, smaller trees or shrubs - probably because of wind pruning (e.g. *Epacris impressa, Leucopogon ericoides, Acacia verticillata, Eucalyptus globulus,* etc.). In sheltered coastal areas, plants may be more robust (e.g. *Cyathodes glauca, Pimelea nivea, Drimys lanceolata,* etc.). Flower size is not necessarily affected but it may accompany vegetative changes (e.g. *Pimelea nivea* - larger flowers).

(a) *E.stuartii*

Although *E.stuartii* is currently recognised as a distinct species, its status as such seems doubtful, and more probably it is a variety of *E.tasmanica*. Evidence to support this may be derived from historical, morphological and ecological observations.

The original description of *E.stuartii* was published by Stapf (1910). From his comments in this paper, one feels that Stapf may not have been thoroughly familiar with the genus *Epacris* in Tasmania. Of the two species he described (*E.stuartii*, and *E.bawbawbiensis = E.paludosa*) he wrote -

> "As to their affinities, it is difficult to pronounce without overhauling the whole genus, but it may be said that at present they will be best placed near *E.myrtifolia* and *E.exerta*."

Although it is not clear which of the two species Stapf intended, close affinities of either with *E.myrtifolia* seem to be somewhat tenuous. Undoubtedly *E.stuartii* may be said to have affinities with *E.exerta* but no more so than with *E.virgata* (or *E.glabella*) and less so than with *E.tasmanica* and *E.barbata*. Since he does not mention *E.tasmanica* (or *E.serpyllifolia* var. *squarrosa* as it was known in 1910), it is unlikely that he had seen this species, and certainly not the range of forms

that it takes. Consequently, on the basis of historical considerations, it seems that *E.stuartii* may have arisen from an inadequate knowledge of other Tasmanian *Epacris* species, and its validity may be questioned.

Curtis (1963) describes the vegetative parts of *E.stuartii* thus -

> "A small shrub resembling *E.squarrosa* (= *E.tasmanica* - Curtis, 1969) and differing chiefly in the leaves which are very shortly stalked, the blade flat, spreading but not recurved, elliptical-ovate to obovate, 4 - 7 mm. long, shortly acuminate to mucronulate, pungent pointed, thick, margin rounded and entire, lower surface 1 - 3 ribbed."

Of *E.squarrosa* (*E.tasmanica*) she writes -

> "Small shrubs ..... . Leaves shortly stalked, broadly lanceolate, tapering into a pungent point, 6 - 10 mm. long or broadly ovate, when shortest, sometimes almost orbicular, acuminate and sharply pointed, 3.5 - 6 mm. long, all leaves spreading and recurved."

The similarity between *E.stuartii* and the short leafed form of *E.tasmanica* is apparent from these descriptions and can be seen in Fig. 17 (p. 124) which shows a comparison between the two species.

The diagnostic character used to separate *E.stuartii* and *E.tasmanica* is recurved versus non-recurved leaves. Not only does this character seem trivial but it shows some variability within populations of both species indicating that under the appropriate selection pressures, the form of this character could change relatively easily in either species.

Several variations in plant form resulting from ecological differences have been mentioned briefly on p. 122. Since these modifications are known to occur, it would not seem illogical to suppose that *E.tasmanica* could also undergo some of these changes if it encountered severe coastal conditions. Differences in habit can be observed between *E.stuartii* and *E.tasmanica*, and these can be related to growing conditions. *E.stuartii* is usually a lower shrub than *E.tasmanica* and tends to be more branched from the base. In general, *E.tasmanica* is more erect, with longer, more slender branches, although its height does tend to vary at different locations. A similar effect between inland and extreme coastal populations is apparent in some other native species e.g. in *Eucalyptus globulus* (but on a grander scale).

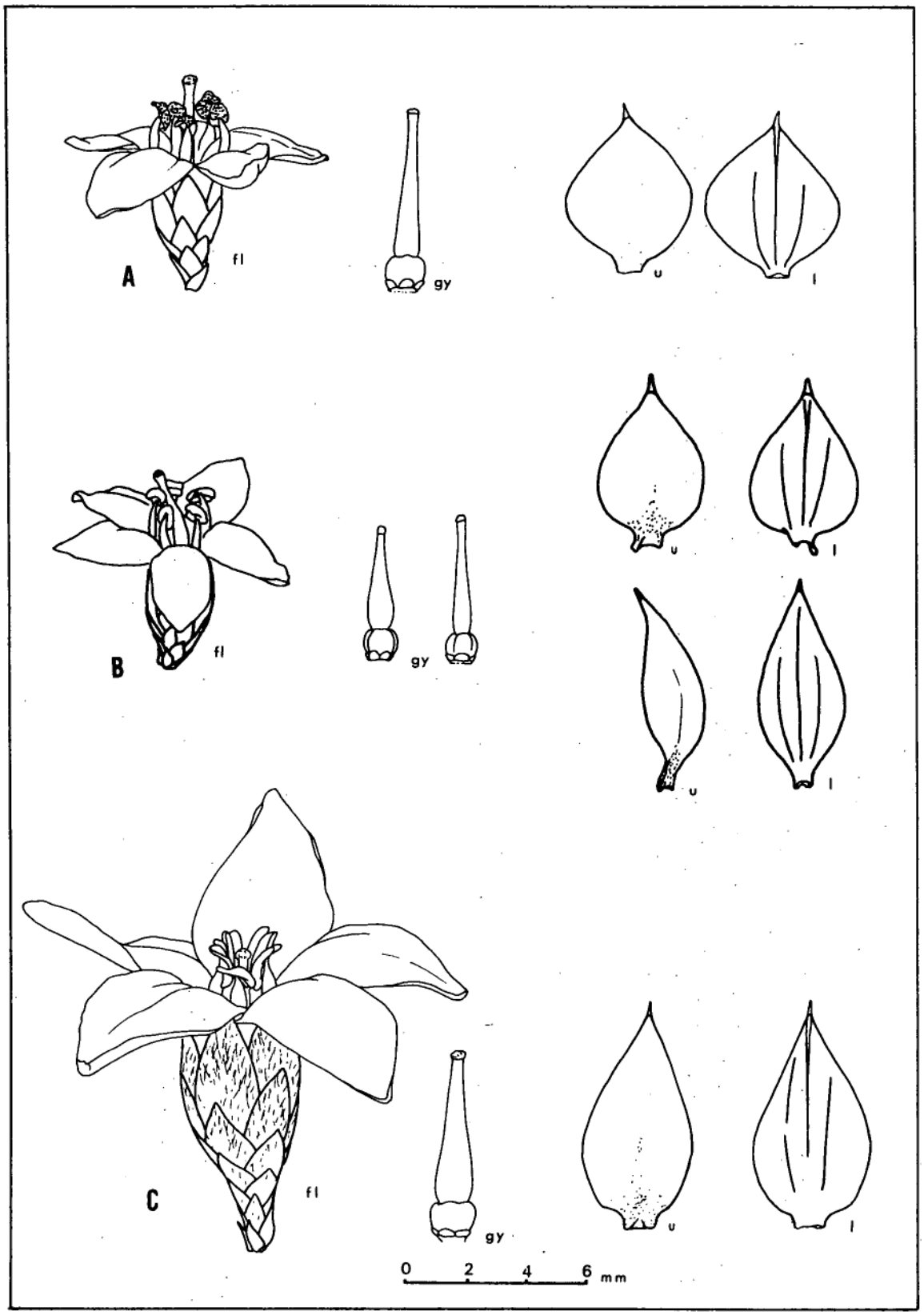


Fig. 17. *Epacris stuarii* (A), *E.tasmanica* (B) and *E.stuartii* (C).
(fl = flower l = lower leaf surface
gy = gynoecium u = upper leaf surface)

With respect to leaf shape, a short, broad leafed form of *E.tasmanica* is known from Tasman Peninsula (see Fig. 17), and it forms a morphological intermediate between *E.stuartii* and the longer leafed *E.tasmanica*. The area in which it grows is near the sea but situated in a very sheltered position on the Peninsula. *E.stuartii*, on the other hand, is known only fron an open coastal heath exposed to the harsh south-easterly gales (Southport Bluff - SE Tasmania). With little imagination, one can visualize the broad leafed form of *E.tasmanica* in an exposed situation appearing very similar to *E.stuartii*.

An examination of flavonoid differences between the two species does little to assist the problem. The flavonol content of *E.tasmanica* leaves is variable along the east and south-east coast (see Fig. 18), and from the more southern locations i.e. Margate, Snug and Eaglehawk Neck, could not be readily separated from those of *E.stuartii*. Anthocyanin and floral flavonol patterns are indistinguishable between the two species. (By itself, this latter observation appears potentially important, but in the context of the whole genus, its significance is lessened by a similar condition occurring among all group E1 species.)

On the basis of information discussed here (historical, morphological, ecological and chemical), and impressions gained in the field, I believe there is sufficient evidence to expand *E.tasmanica* to include *E.stuartii*. *E.tasmanica*, as currently recognised, is a very variable species morphologically (and chemically) and the inclusion of *E.stuartii* would not cause any discontinuity in this variability. However, the union of the two species is not imperative at the moment, and I prefer to leave them separate until as many avenues of research have been explored as possible e.g. anatomy, seed protein chemistry and growth experiments.

(b) *E.barbata*

This species is readily separated from other *Epacris* species by its diagnostic character (densely hairy sepals). However, it is interesting to speculate on its possible ancestry.

E.barbata grows in warm, relatively protected heaths on Freycinet Peninsula (east coast) where it is found associated with granite soils. It resembles *E.tasmanica* in form but the shrub is more robust and the flowers are generally larger. In addition, the style is very bulbous in the lower half, and comparatively short in relation to the length of the calyx. While this contrasts with the typical form of *E.tasmanica*,

SOUTH EASTERN TASMANIA

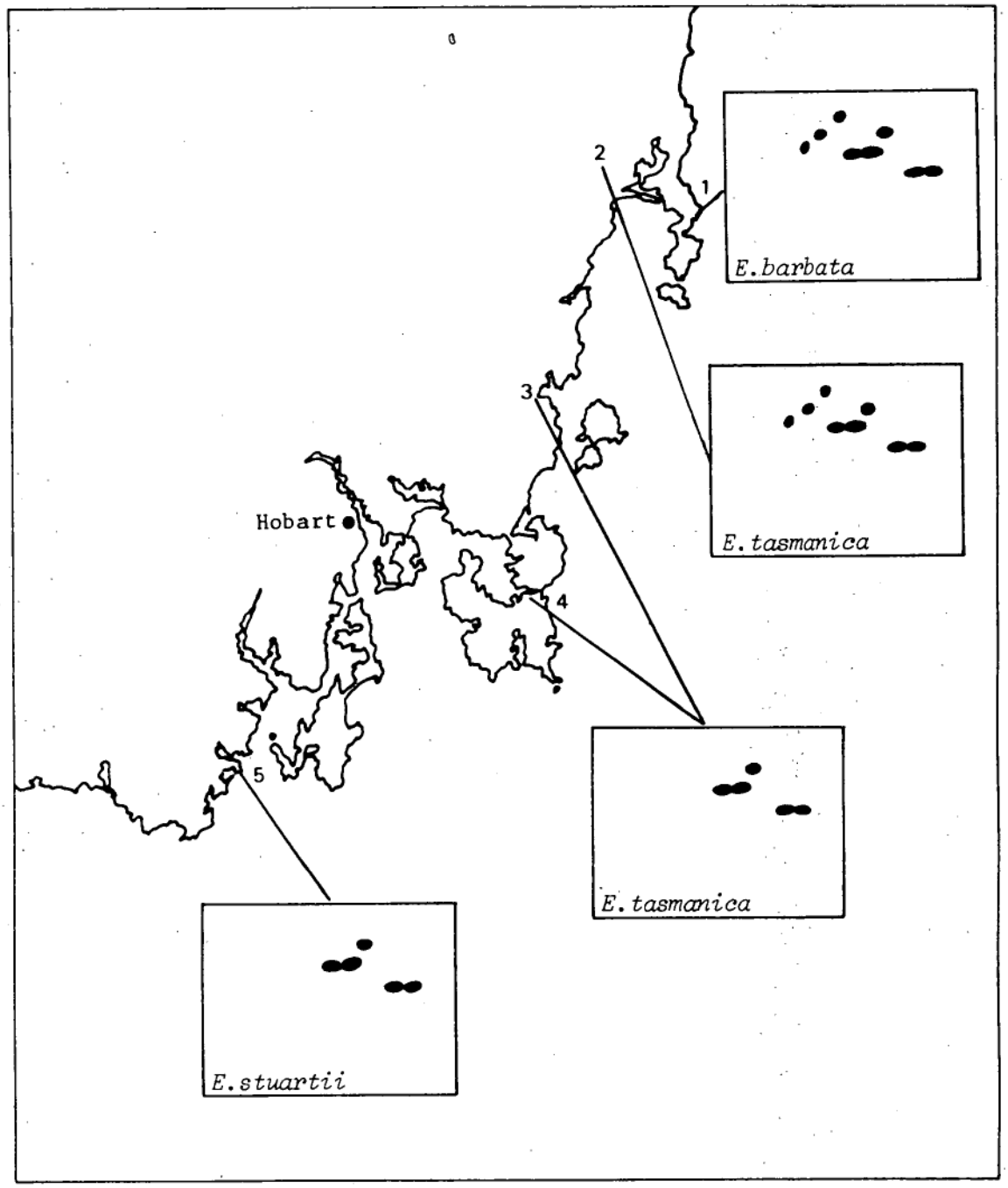


Fig. 18. Leaf flavonol patterns of *E. tasmanica* and related species.

(1 = Coles Bay
2 = Cranbrook
3 = Orford
4 = Eaglehawk Neck
5 = Southport Bluff)

a population at Cranbrook (E. coast) was found to resemble *E.barbata* in this manner.

It seems probable that the effect of nearness to the sea and a different soil type have combined to produce a larger form of *E.tasmanica* which has been called *E.barbata*. This view is supported by the nearby occurrence of *E.tasmanica* (within ten miles) and its absence on Freycinet Peninsula even where the right type of habitat (apparently) is present. In addition, at least two other species are known to produce a larger, more vigorous plant in coastal areas (see p.122) so the possibility that *E.tasmanica* may do likewise seems quite probable.

In chemical characters, *E.barbata* intergrades with the more northern forms of *E.tasmanica* (see Fig. 18). In the population of *E.barbata* studied at Coles Bay, four plants were characterized by the presence of myricetin derivatives whilst these were absent in a fifth plant. This situation intergraded with that apparent in *E.tasmanica* populations where myricetin derivatives were present in some (northern populations) but not in others (southern populations).

In all probability, *E.barbata* has been derived from *E.tasmanica* and is still sufficiently similar to be considered a variety, if consistency in taxonomic procedure were to be maintained. However, since its diagnostic character (hairy sepals) can be used to circumscribe all plants in a given area, and since there is no practical advantage to be gained from uniting *E.barbata* and *E.tasmanica*, it is less confusing to leave the two separate.

A morphological comparison between *E.tasmanica* and *E.barbata* is shown in Fig. 17 (p. 124).

(c) *E.glabella*

The description of *E.glabella* is given on p. C8 (Appendix C). It is a new species, closely allied to *E.tasmanica*, *E.barbata*, *E.stuartii*, *E.virgata* and *E.exerta* with whom it shares similar floral characters. It differs from these species in having a combination of glabrous branchlets, shiny leaf surface (upper surface), blunt leaf apex and ovate shaped leaves (see Fig. 19, p. 128).

The inclusion of *E.glabella* in group E1 is supported by chemical evidence. The flavonol pattern in the petals is consistent with that found in species of this group and in addition, Qu-3-Gal, Qu-3-Glur, Qu-3-Arab and Km-3-Rha are present (in common) in the leaves. The presence of Km-3-Gal and Km-3-Glur is uncertain in the leaves of *E.glabella*.

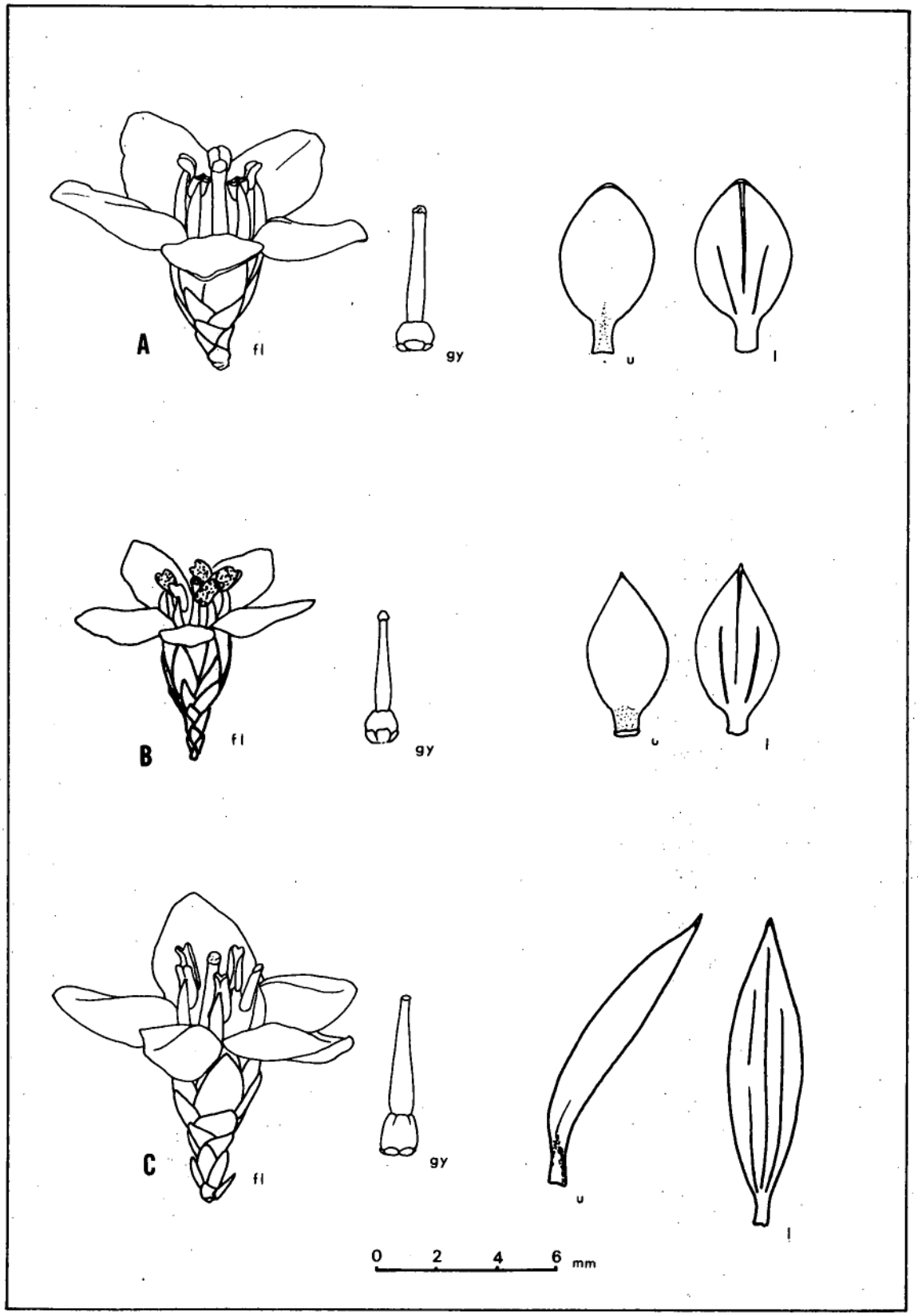


Fig. 19. *Epacris glabella* (A), *E.virgata* (B) and *E.exerta* (D).
(fl = flower l = lower leaf surface
gy = gynoecium u = upper leaf surface)

(d) *E.heteronema*

In the following discussion, flowers have not been considered since they show little variation in the field and their description, taken from several sources, is consistent.

The original description of *E.heteronema* (Labillardiere - 1804) is very brief. The vegetative parts are described thus -

> "*Epacris* foliis ovata-oblongis, acuminatis; ..... . *Frutex* sesquipedalis. *Folia* subpetiolata, dorso striis sex octo notata, in ramulis hirsutis imbricata."

With one exception, the characters mentioned here could describe any one of several species. The diagnostic character, "*Folia* .... dorso striis sex ad octo notata", in combination with floral characters, completely separates *E.heteronema* from other *Epacris* species. Although R.Brown (1810) and J.D.Hooker (1860) also describe the leaf undersurface of *E.heteronema* as being striate, following descriptions (Bentham - 1869, Rodway - 1903, Curtis - 1963) fail to mention this character. Indeed, Bentham is quite contradictory and describes the leaves as "almost veinless in the typical form". As well as this typical form, Bentham also describes a variety, var.? *planifolia* of which he writes "..... the midrib and sometimes the lateral veins conspicuous beneath". However, Bentham does not list specimens from Recherche Bay (southern Tasmania) under this variety but includes them in the typical form. From my own observation, specimens from the Recherche Bay area are conspicuously striate. In fact, it is from this locality that Labillardiere obtained his plants.

The apparent contradiction in Bentham's description may be due to the composite nature of his species for in 1910, Stapf divided it into 4 species, one of which he refers to Labillardiere's original *E.heteronema*. In his revised description of this species, Stapf describes the venation as "..... nervis tenuibus saepe obscuris subparallelis". Whether this is intended to mean "striate" is difficult to say but certainly the remainder of his description does not conflict with Labillardiere's although it gives more detail. He was apparently familiar with Labillardiere's specimens for he writes in his paper (1910), "One of Labillardiere's original specimens is at Kew and there can be no doubt as to the plant which he meant". Stapf's description, in turn, does not conflict with that given by Curtis (1963) and in this thesis,

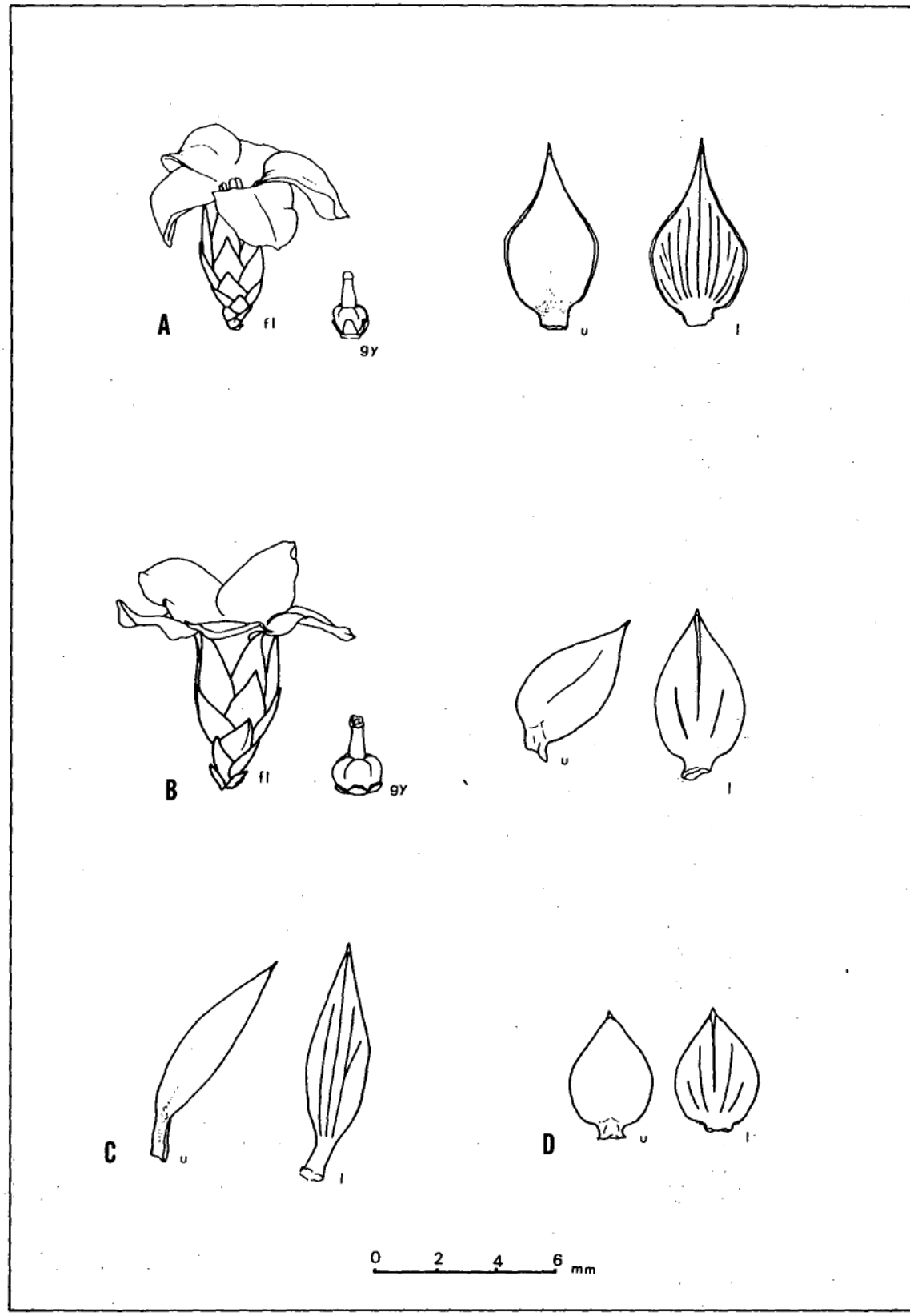


Fig. 20. *Epacris heteronema* (A), *E.* var. *Davies Ck.* (B), *E.mucronulata* (C) and *E.serpyllifolia* (D).

(fl = flower
gy = gynoecium
l = lower leaf surface
u = upper leaf surface)

the latter will be followed, with one slight modification. An additional character and in my opinion, the diagnostic one, should be inserted in the description i.e. "leaf undersurface striate with 5 or more parallel veins". Hence, the description of *E.heteronema* Labill. would read -

> "A stout, much branched shrub frequently 30 - 100 cm. high but sometimes (on the west coast), a small tree up to 6 m. high. Leaves with short wide stalks, crowded and often imbricate, spreading or ± erect; blade 6 - 12 mm. long, variable in shape, ovate or broadly ovate-cordate or narrow ovate, acuminate and pungent pointed, margin narrowly hyaline and minutely serrulate, thick, flat or concave, undersurface striate with five or more parallel veins. Flowers solitary ..... (as in Curtis - 1963).".

As yet, I have seen no specimens with cordate leaves but apparently this is a variable character in the species.

A drawing of *E.heteronema* collected from Southport (southern Tasmania) is shown in Fig. 20.

(e) *E.mucronulata*

Specimens of *Epacris* collected from the Picton River (southern Tasmania) have been identified as *E.mucronulata*, but since they do not key out as this species, some further explanation is considered necessary.

In the original description of *E.mucronulata* (= *E.franklinii* Hook.), Hooker places the species amongst those whose apex is "acute or acuminate but not pungent". In my opinion, the leaf apex of plants from the Picton River is sharp, although not pungent. This prevents their identification as *E.mucronulata* from the identification key which is currently available in Tasmania (Curtis - 1963). In this key, they are obliged to agree with "apex acute or mucronate but not sharp" in order to be identified appropriately. Without question, the distinction between "sharp" and "not sharp" is very subjective particularly in cases such as this, where the extreme character states do not occur. Consequently, I feel that the exclusion of Picton R. specimens from *E.mucronulata* on this basis is unrealistic. My own experience of "typical" *E.mucronulata* (with a blunt leaf apex) is limited to herbarium specimens but these are very similar in general appearance to those plants obtained from the Picton R.

(f) *E.* var. *Davies Creek*

Two forms of this species are known, one with short broad leaves (see Fig. 20, p. 130), and one with longer, narrower leaves. Both were growing together on Mt. Arrowsmith Pass (west coast). Its description is given on p. C8 (Appendix C).

E. var. *Davies Ck.* keys out unsatisfactorily as *E.lanuginosa* (Rodway - 1903) but it can be separated immediately from this species by the absence of a hairy style (diagnostic of the latter species). It also keys out as *E.heteronema* (Bentham - 1869, Curtis - 1963) and although the two are similar in floral morphology, they differ in leaf venation, leaf shape and general aspect of the plant.

The two species with which *E.* var. *Davies Ck.* shows the most similarity are *E.serpyllifolia* and the sharp leafed *E.mucronulata* found at the Picton R. (see p. 130). *E.* var. *Davies Ck.* appears to be more or less intermediate between the two, being linked to the former through the short broad leafed form and to the latter by the narrow leafed form. However, it does not key out as either because, like *E.mucronulata*, the leaf apex is sharp (see Curtis - 1963) and the sepals are ciliate (see Rodway - 1903).

With respect to plant habit, *E.* var. *Davies Ck.* is similar in shrub height (up to 2 m.) to *E.mucronulata*, but differs from *E.serpyllifolia* which is recorded as "about 1 foot" (Rodway - 1903) or "up to 1 m." (Curtis - 1963). However, height tends to be a very variable character and in the relatively milder conditions (sheltered rainforest) one would expect *E.* var. *Davies Ck.* to be taller than "typical" *E.serpyllifolia* (from alpine conditions).

With floral characters, differences between *E.* var. *Davies Ck.* and both *E.mucronulata* and *E.serpyllifolia* are of the same order, excepting that style length is variable in *E.mucronulata.*

An examination of flavonoid chemistry does little to assist the problem since it tends to reflect the close morphological similarities between the species. Chemical variation between the three is generally restricted to minor constituents and is probably quantitative in nature. The occurrence of D_{37} in the leaves of *E.serpyllifolia*, D_{34} in *E.mucronulata* and both in *E.* var. *Davies Ck.* would suggest the intermediate nature of the latter species. This is also apparent in the flowers, where My-3-Gal is present in *E.serpyllifolia*, D_{34} in *E.mucronulata* and both in *E.* var. *Davies Ck.* However, since the occurrence of these compounds is variable in each species, it would

seem likely that if enough populations were examined, all compounds would be detected in all species.

From this research, the taxonomic status of *E.* var. *Davies Ck.* is not clear. Its intermediate nature between *E.serpyllifolia* and *E.mucronulata* is apparent from both morphology and chemistry. Consequently, until further evidence comes to hand, *E.* var. *Davies Ck.* will be maintained separately from both.

The short broad leafed form of *E.* var. *Davies Ck.* is shown in Fig.20 and the leaves of *E.serpyllifolia* are *E.mucronulata* included for comparison.

(g) *E.impressa*

This species is widespread throughout the state and is variable in form. Hooker (1860) listed two additional species to *E.impressa*, i.e. *E.ceraeflora* and *E.ruscifolia*, both of which he considered doubtfully distinct. Rodway (1903) includes these species as varieties of *E.impressa* and while Curtis (1963) describes no varieties, she writes -

> "A polymorphic species, the plants varying in colour and size of flowers and in shape and size of leaves; several variants have been given varietal or specific rank.".

In all variants, the general morphological appearance of the flowers is similar (with 5 impressions at the base of the tube). Chemical patterns among the different forms are consistent, with the exception of gradations in anthocyanin concentration between white and pink flowers. Leaves are characterized by two novel pigments found elsewhere only in 2 distinct mainland species.

(h) *E.gunnii*

E.gunnii is listed as endemic to Tasmania (Curtis - 1963) but accessions from the mainland indicate quite clearly that this species is present in the Blue Mountains of New South Wales. Chemical data does not dispute this view but so few compounds are accumulated that no significant conclusions can be derived from it.

B. *CYATHODES*

Nine species of *Cyathodes* have been recorded from Tasmania (Curtis - 1963, Jarman - 1974). One species, *C.juniperina*, occurs in Victoria and New Zealand but the remaining species are endemic. They occupy predominantly alpine or rainforest habitats although one species, *C.divaricata*, regularly grows in lowland Eucalypt forests of the east and south-east whilst another, *C.abietina*, occurs only along the sea shore, on exposed windswept coasts. The genus is found throughout the state although it is uncommon in coastal areas of the north and north-east.

The plants vary in form, from low or prostrate shrubs, to medium shrubs, and in southern and western areas one species, *C.juniperina*, may form a small tree up to 10 m. high. The flowers are small, white or cream, and usually solitary excepting in *C.petiolaris* where two flowers are occasionally present in the leaf axils. In most species, the fruit is crowded on the plant and excepting in *C.dealbata*, usually persists for several months. It is less than 1 cm. in diameter but is conspicuously coloured (pink, red, purple) by anthocyanin pigments. The mesocarp is white and pulpy in most species but is watery in *C.dealbata*. Several species are dioecious.

On the basis of morphological similarity, three groups (C1, C2 and C3 - for convenience) are apparent in the genus. The constituent species and the characters used to define the groups are shown in Table 37 (p.135). The position of *C.abietina* is in some doubt since it shares characters with species from both C1 and C2. For example, in fruit shape, size and colour, and leaf morphology, it resembles C1 species but is similar to species of C2 in its breeding system. In addition, the corolla lobes are densely bearded as in *C.petiolaris* (C2).

Apart from *C.abietina*, all three groups are readily separated by the characters listed. *C.dealbata* has not been included in any of the three groups and its 'peculiarities' are discussed on p. 145.

The three groups are not clearly delimited by chemical data. In group C3, there appears to be a greater tendency towards the accumulation of flavonoid biosides than in the other groups, and it is the only group to accumulate delphinidin and myricetin 3-biosides. Alternatively, C1 is the only group in which D_{30} and D_{34} are found, but the occurrence of these compounds is not consistent.

Results from the numerical analyses using chemical data (see p.107)

Group C1

C.parvifolia, C.juniperina, C.divaricata, C. var. *pendulosa, (C.abietina)*

Morphological characters

(i) Dioecious or partially so.
(ii) Corolla lobes glabrous or with a few scattered hairs.
(iii) Fruit rounded, pink-red.
(iv) Leaf undersurface with 3 - 5 parallel viens.
(v) Leaf apex with a slender pungent point.
(vi) Leaves lanceolate.
(vii) Shrub erect.

Group C2

C.petiolaris, C.nitida, C. var. *intermedia*

Morphological characters

(i) Hermaphroditic.
(ii) Rudimentary bud present on the pedicel.
(iii) Fruit flattened, red or black-red.
(iv) Highland species.
(v) Low shrubs with branches spreading and the tips ascending.

Group C3

C.straminea, C.glauca

Morphological characters

(i) Hermaphroditic.
(ii) A few scattered hairs on the corolla lobes.
(iii) Leaves in annular clusters.
(iv) Leaf undersurface with more than 7 veins.

Ungrouped species

C.dealbata

Table 37. Major divisions in *Cyathodes*, based on morphological characters.

lend some support to the morphological divisions suggested here. Apart from *C.abietina* and *C.* var. *pendulosa*, species are grouped according to the arrangement given in Table 37. The position of *C.* var. *pendulosa* varies with the two classifications constructed, being placed in C1 according to CLASS and in C2 according to MULTBET. The position of *C.abietina* is consistent in group C2 in both classifications.

Possible reasons to account for the difference between numerical results and those obtained from subjective analyses have been given on

p. 121 for *Epacris* and the same applies here. For this reason, in this section, the arrangement based on morphological data will be followed.

1. Comments on some species

(a) *C.juniperina* and *C.parvifolia*

C.juniperina is a widespread, variable species, ranging from coastal areas where it may form a small tree (up to 10 m.) to highland areas where it grows as a small shrub similar to *C.parvifolia*. As well as shrub height, leaf size decreases towards higher altitudes. *C.parvifolia* is restricted to highland areas, and is typically a low shrub (less than 1 m. high) with leaves shorter than 0.6 cm.

The two species are traditionally separated by leaf and shrub size, and differences in the pedicel and the breeding system. However, the reliability of these characters is frequently in doubt. Rodway (1903) uses characters of the pedicel ('recurved' versus 'very short') in combination with leaf characters to separate the two. However, neither Bentham (1869) nor Curtis (1963) has recorded any significant difference in the pedicel and from my own experience, the character seems variable and may be related to plant sex - male flowers usually having a longer pedicel than female flowers. Curtis (1963) attempts to separate the two species using differences in the breeding system (also in combination with leaf characters). *C.parvifolia* is described as dioecious and *C.juniperina* as hermaphroditic. For the latter species, populations at Eaglehawk Neck, Lake Fenton, Dundas Road and Mt.Rufus canal all appear to be dioecious (or gynodioecious). I have not seen any populations of *C.juniperina* which appear to be hermaphroditic.

The two most favoured characters used to separate the species are leaf length and shrub height. In very different habitats, these characters can be used easily and with confidence, but under similar environmental conditions, their convergence in the two species precludes their effective use. Because of this variation, the identity of *C.juniperina* and *C.parvifolia* cannot always be determined with certainty. In areas of overlapping distribution, it is usually possible to distinguish two forms but the distinction is often subtle and rests with slight differences in leaf orientation and shrub density, neither of which are consistent. Dried material, particularly of specimens from higher altitudes, is usually very difficult to identify.

Under these circumstances, the validity of each species as a separate entity may be questioned. Rodway (1903) was apparently

uncertain, and although he describes *C.parvifolia* separately, he remarks that it is doubtfully distinct from *C.juniperina* and *C.divaricata*. Sleumer (1963), without indicating the reason, includes *C.parvifolia* as a variety of *C.juniperina*. Other taxonomists, while admitting to the close affinity between the two species, seem happy that they should remain separate, at least for the present.

A morphological comparison between the two species is shown in Fig. 21 (p. 138).

An examination of flavonoids in the two species suggests that chemistry may offer some useful contribution to the problem, at least in terms of identification. Three distinct differences occur between the two species.

C.juniperina consistently produces two flavonol biosides (D_{26a} and D_{26b}) in all tissues examined whilst both pigments are absent from *C.parvifolia* tissues. From Mt.Field, where the distribution of the two species overlaps and morphological characters intergrade, the identity of plants tentatively assigned to either species on the basis of slight differences in leaf density and orientation (see p. 136) were confirmed by the chemical data. However, there is the possibility that plants identified as *C.juniperina* may in fact be hybrids between *C.parvifolia* and *C.juniperina*, and in favouring the morphological form of the latter parent, may also carry the diagnostic chemical characters. That hybridization between the two does occur in the area is suggested by the intermittent presence of Qu-3-RhaGlc in *C.parvifolia* leaves and fruit. This pigment is the dominant pigment in *C.juniperina* leaves and fruit but is not normally detected in *C.parvifolia*.

The second distinct difference between the two species is associated with the accumulation of dominant flavonoid constituents. In *C.juniperina* populations (with one exception) a bioside (Qu-3-RhaGlc) is accumulated as the most concentrated flavonol glycoside whilst in *C.parvifolia*, a monoside (Qu-3-Glur) is consistently the dominant glycoside. In one exceptional population of *C.juniperina* (from Eaglehawk Neck) the dominant flavonol is also Qu-3-Glur, as in *C.parvifolia*. However, in this area, *C.juniperina* is morphologically distinct (leaves >1 cm., shrub height >1.5 m.) and could not be confused easily with *C.parvifolia*. The change in glycoside dominance in this population may be associated with habitat differences and the same parallel behaviour is also apparent in the fruit anthocyanins i.e. highland populations accumulate Cy-3-RhaGal in excess of Cy-3-Gal whereas

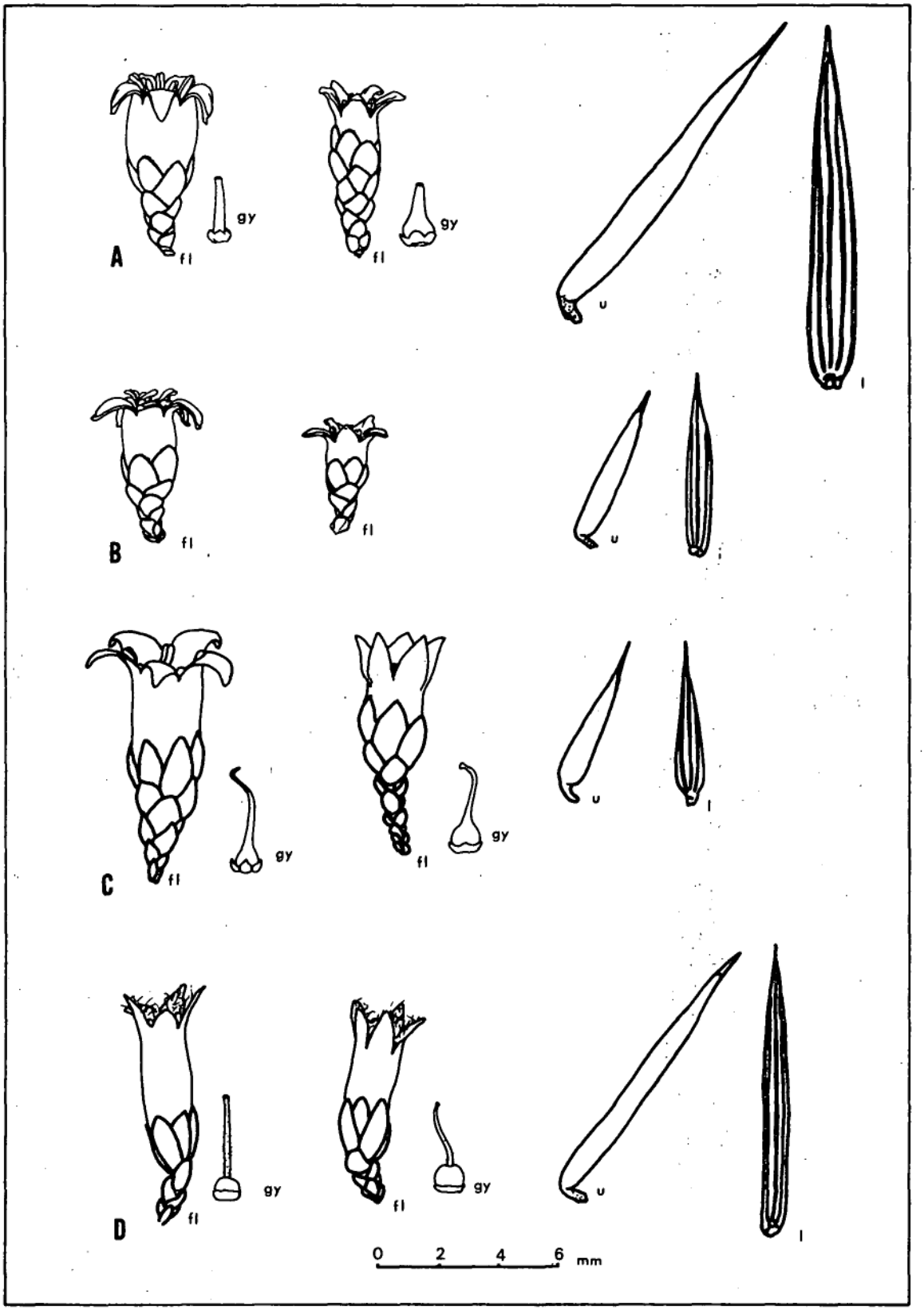


Fig. 21. *Cyathodes juniperina* (A), *C.parvifolia* (B), *C.* var. *pendulosa* (C) and *C.divaricata* (D). (Male and female flowers are present for each species.)

(fl = flower l = lower leaf surface
gy = gynoecium u = upper leaf surface)

the reverse situation occurs in the coastal population at Tasman Peninsula.

The third clear chemical difference between the two is associated with the accumulation of two dark absorbing spots on chromatograms of fruit tissues. The identity of these compounds has yet to be established but their behaviour appears to be consistent in all populations examined. *C.parvifolia* typically accumulates both compounds whilst *C.juniperina* accumulates only one.

At this stage, chemical data would suggest that two species are present which, under the appropriate circumstances will hybridize. The chemical differences between the two (listed above) should prove valuable in examining the possibility of hybridization.

(b) *C.divaricata*

The identification of *C.divaricata* is usually straightforward because of its cream coloured flowers and the scattered hairs on the corolla lobes. Its close affinity with *C.juniperina* was recorded by Hooker (1860), Bentham (1869) and Rodway (1903). Rodway makes the following note -

> "The species bears no character of importance differing from *C.acerosa* (= *C.juniperina*) and doubtless should not be considered more than a variety of that species; but the tendency for the bracts to be reduced in number, leaving a portion of the peduncle bare showing its close relationship to *Lissanthe*, perhaps warrants its retention.".

It is of interest that *C.divaricata* differs in chromosome number (n = 12) from the closely related species, *C.juniperina* and *C.parvifolia*, where the haploid number is 10 (Smith-White - 1955). Chromosome numbers are reported as n = 7 and 14 for *Lissanthe* (Smith-White - 1955).

(c) *C.* var. *pendulosa*

C. var. *pendulosa* appears closest to *C.parvifolia* and *C.divaricata* and although it does not fit exactly into the typical form of these species, with little imagination, it could be included as a variety of either. It was found growing in open Eucalypt forest on the foothills of Ben Lomond (north-eastern Tasmania).

In the available floral keys (Bentham - 1869, Rodway - 1903, Curtis - 1963), *C.* var. *pendulosa* is identified as *C.parvifolia* but this is due to the emphasis placed on the presence or absence of a few long scattered

hairs on the corolla lobes.

C. var. *pendulosa* is similar to *C.divaricata* in habit but closely resembles *C.parvifolia* in leaf size. The flowers are white with glabrous lobes similar to *C.parvifolia* but are larger, attaining the shape and size of *C.divaricata* flowers. Loose, scarcely imbricating bracts are present on the pedicels of male flowers (similar to both male and female flowers of *C.divaricata*) but the bracts of female flowers are more crowded like those of *C.parvifolia*. The fruit shape and size are more similar to those of *C.divaricata*.

The distribution and altitude range of *C.* var. *pendulosa* and the length of its flowering season are not known. It was growing in association with, but was readily distinguishable from, *C.parvifolia* at the lower altitude limit of this latter species. *C.divaricata* was not apparent in the area but the habitat appeared suitable (rocky, open *Eucalyptus* forest) and it seems possible that it is replaced here by *C.* var. *pendulosa*. Flowers were present when first collected in early May (by Mr.R.Shepherd) and also in late June when collected by Dr.R.Crowden and myself. On this limited information, *C.* var. *pendulosa* appears to be more typical of *C.divaricata* in its flowering season.

Similarities between *C.* var. *pendulosa*, *C.parvifolia* and *C.divaricata* are apparent from an examination of flavonoid compounds. A summary of the results is shown in Table 38 (p. 141). Both *C.parvifolia* and *C.divaricata* exhibit intraspecific chemical variation and since the latter species does not apparently occur in the area, chemical differences between it and *C.* var. *pendulosa* may be exaggerated by differences in response to the habitat. A similar situation occurred with flowers of *C.parvifolia* which were not available from Ben Lomond at the time. Hence, it is possible that the comparison shown in Table 38 underestimates the similarity between the three.

From the chemical results, *C.* var. *pendulosa* shows no clear cut bias towards either *C.parvifolia* or *C.divaricata* but has several characteristics in common with one or both species. Along with morphological differences (see Fig. 21, p. 138), this may indicate that *C.* var. *pendulosa* is worthy of species status. However, further collections are required before any decision can be made.

The origin of *C.* var. *pendulosa* through hybridization between *C.parvifolia* and *C.divaricata* seems possible. *C.parvifolia* has a short flowering season in spring which is overlapped by the extended season of *C.divaricata* (late autumn to summer). Hence, interbreeding between the

Leaves	*C.v.pen*	*C.parvi*	*C.div.*
Qu-3-Arab, Qu-3-Rha	+	+	+
Qu-3-Gal, Qu-3-Glur	+	+	+
Km-3-Rha	+	+	+
D_{30}	+	+	+
Km-3-Glur	-	+	+
My-3-Arab	-	+	+
My-3-Gal	-	+	+
My-3-Glur	-	+	+
Qu-3-RhaGlc	-	+*	-
D_{34}	-	+	+
Flowers			
Qu-3-Glur, Km-3-Rha	+	+	+
Qu-3-Gal	-	+	+
Qu-3-Rha	+	+	-
Km-3-Glur	+	-	+
My-3-Glur	-	+	+
D_{30}	-	+	+
D_{34}	-	+	+
Fruit			
Qu-3-Glur, Qu-3-Rha	+	+	+
My-3-Glur	+	+	+
Br_2	+	+	+
Qu-3-Gal	-	+	+
Km-3-Glur	+	-	-
Km-3-Rha	+	-	-
Qu-3-RhaGlc	-	+*	-
D_{30}	-	-	+
D_{34}	-	-	+
Br_1	-	+	-

Table 38. A comparison of flavonols in *C.* var. *pendulosa (C.v.pen)*, *C.parvifolia (C.parvi)* and *C.divaricata (C.div)*.

* Not present in the Ben Lomond population.

two would not be prevented by a separation in critical developmental stages. The distribution of *C.parvifolia* and *C.divaricata* is also known to overlap e.g. Lake Leake (north eastern Tas.), although no intermediate plants were observed in this area. If interbreeding did occur, the possibility arises that the hybrid, inheriting adaptive characteristics from both parent species, would be more suited to the intermediate habitat than one (or both) of the parents, in this case *C.divaricata*. It may have been able to compete successfully with *C.divaricata* to result in the gradual exclusion of this species from the area.

Although this hypothesis is purely speculative, it does have some

supporting evidence in the close similarity between the two "parent" species, their overlapping distribution and flowering time, and the morphologically intermediate nature of the possible "hybrid".

A description of *C.* var. *pendulosa* is given on page C7.

(d) *C.* var. *intermedia*

C. var. *intermedia* is intermediate in its morphological characters between *C.petiolaris* and *C.nitida* (see Fig. 22, p. 143 - a description is given on p. C6, Appendix C). Leaf characters approach those of *C.nitida* with similarities in shape, the recurved margin and the number of veins apparent on the undersurface. However, the undersurface is glaucous (similar to *C.petiolaris*) and the leaf length is greater (up to 1.5 cm.). The corolla lobes are densely bearded, but the outer third may be quite glabrous. In *C.petiolaris*, the lobes are bearded for their whole length and in *C.nitida* they are usually glabrous although occasionally a few inconspicuous hairs may be present. The fruits are similar in size and shape to those of *C.petiolaris* but differ in being completely glabrous.

In overall appearance, *C.* var. *intermedia* is more similar to *C.petiolaris*. It is even possible to find "juvenile" leaves on *C.petiolaris* (towards the base of the branches) which are similar to those present at maturity in *C.* var. *intermedia*. This could suggest that the characteristics of the juvenile leaves in *C.petiolaris* have been retained by the varietal form, *C.* var. *intermedia*, to constitute the adult foliage.

In her description of *C.petiolaris*, Curtis (1963) mentions a form known from the west coast mountains and presumably *C.* var. *intermedia* is this form. It seems to be restricted to the west and south west of the state where it may be replacing *C.petiolaris*. On two mountains where I have seen *C.* var. *intermedia* growing (Mt.Hamilton and Frenchman's Cap), there was no sign of *C.petiolaris*, even though habitat conditions appeared suitable.

With regard to chemical characters, *C.* var. *intermedia* appears to accumulate fewer flavonols in the leaves and flowers than either *C.nitida* or *C.petiolaris* and those that are present, are common to all three. In the fruits, the presence of Qu-3-Rha and the absence of flavonol 3-glucuronides differed from both *C.petiolaris* and *C.nitida*. With respect to anthocyanins in the fruit, the accumulation of anthocyanin arabinosides in greater concentrations than anthocyanin galactosides was consistent with the situation in *C.petiolaris* (but not *C.nitida*).

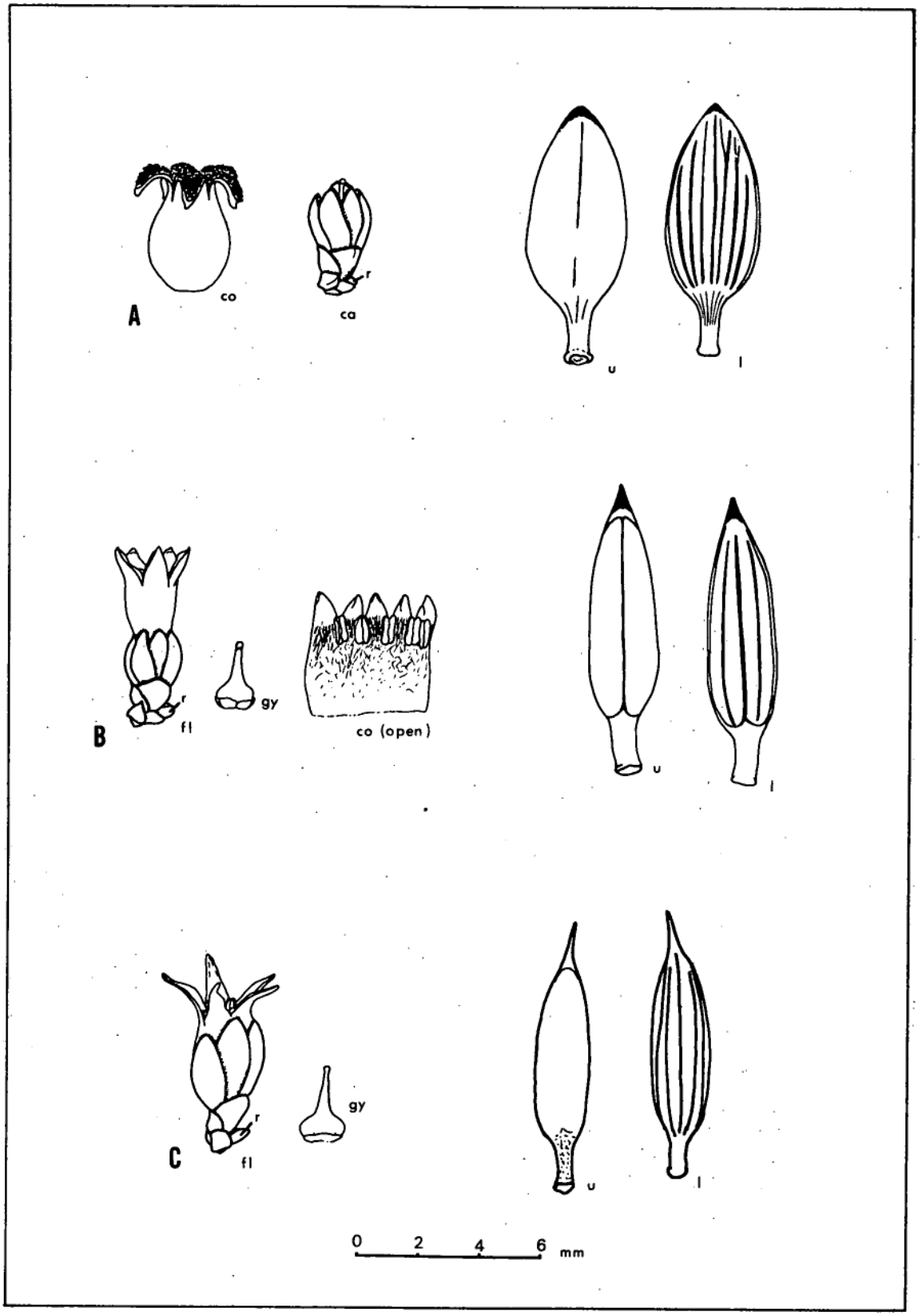


Fig. 22. *Cyathodes petiolaris* (A), *C.* var. *intermedia* (B) and *C.nitida* (C).

(ca = calyx fl = flower l = lower leaf surface
co = corolla gy = gynoecium u = upper leaf surface
r = rudimentary bud)

However, these chemical results require confirmation since all tissues of *C.* var. *intermedia* had been unavoidably subjected to drying out during their transportation to the laboratory.

(e) *C.nitida*

C.nitida is a new species discovered during this research. Its description is given on p. C5 and drawings are shown on p. 143.

C.nitida is readily distinguished from other species of the genus in Tasmania by the very flattened, glossy, black-red drupe along with the non glaucous undersurface of the leaf. Its closest affinities are with *C.petiolaris* and *C.* var. *intermedia*, and together the three occupy an intermediate position between the genera *Cyathodes* and *Leucopogon*. In all three, the flower is solitary (although sometimes 2 or 3 are present together in *C.petiolaris*) and almost sessile. It is subtended by one bract and two bracteoles as in the genus *Leucopogon*. In addition, a rudimentary bud is present terminating the inflorescence. This structure is unknown in other *Cyathodes* species but is commonly found in species of *Leucopogon*. Because of its small size, the rudimentary bud is pushed to one side and appears axillary on the inflorescence. If it is removed, its subtending bract overlaps the bracts of the properly formed flower. The peduncle then appears as a multibracteate pedicel typical of the genus *Cyathodes*. Of *C.petiolaris*, Bentham (1869) writes -

> "This species has the inflorescence of *Leucopogon*, and ought, perhaps, to be transferred to that genus; but the fruit is much more pulpy than in any *Leucopogon*, and the aspect and foliage are quite those of *Cyathodes*."

This statement is also applicable to *C.nitida* (and to *C.* var. *intermedia*) whose position in the genus *Cyathodes* is strengthened by the absence of bearded corolla lobes which are typical of *C.petiolaris* and the genus *Leucopogon*.

C.nitida is similar in habit to *C.petiolaris* and *C.* var. *intermedia* and occupies a similar altitude range (although they have not been found together in the same plant community). In leaf shape, *C.nitida* is similar to *C.* var. *intermedia* (but not *C.petiolaris*) and differs from both in the texture of the undersurface. Other differences include the colour and surface texture of the fruit.

(f) *C.dealbata*

C.dealbata is unusual among Tasmanian species of the genus in

producing a watery, succulent fruit. In other Tasmanian *Cyathodes*, the mesopcarp is pulpy, with virtually no juice.

C.dealbata is chemically distinct, accumulating Cy-3-XylGal and Cy-3-XylArab in the fruit. In addition, it appears to completely lack flavonol 3-glucuronides in the flowers, leaves and fruit, whereas all others with the exception of *C.* var. *intermedia*, accumulate these in one or other of the tissues mentioned. (The results of *C.* var. *intermedia* require repetition - see p. 144). *C.dealbata* consistently accumulates My-3-Rha in significant quantities, and in the flowers and fruit, this is the dominant or co-dominant pigment. In other Tasmanian *Cyathodes*, My-3-Rha is accumulated at conspicuously low concentrations or is absent. Furthermore, *C.dealbata* is the only species that consistently accumulates myricetin derivatives in excess of quercetin derivatives in all tissues studied.

From the chemical evidence obtained during this study, the inclusion of *C.dealbata* in the genus appears doubtful. However, with respect to morphological characters, this species is not out of place apart from its succulent fruit, although several characters are at the extreme edge of the morphological variation found in the genus (see Table 39).

From an examination of the dissimilarity matrices obtained from the numerical analyses (p. B14), it is interesting to note that the smallest dissimilarity value for *C.dealbata* (with *C.* var. *intermedia* - MULTBET, and *C.nitida* - CLASS) is larger than the smallest value for any other two species i.e. *C.dealbata* is at the edge of the genus. This is also apparent from the classifications shown on p. 108. These results support the morphological and chemical data given in Table 39.

Succulent fruit of the type formed by *C.dealbata* is evident in the related genus, *Leucopogon*. The occurrence of an unusual anthocyanin (Cy-3-XylArab) in *C.dealbata* and one species of *Leucopogon* also suggests a relationship between the two. However, they have little else in common, either chemically or morphologically, and at this stage there is no strong evidence to indicate that *C.dealbata* would be better placed in *Leucopogon* than in *Cyathodes*.

Rodway (1903) remarks that *C.pumila* from New Zealand is probably identical with this species, and although descriptions of the two are similar (Curtis - 1963 cf. Allan - 1961), it would be unrealistic to comment without first examining the New Zealand plant.

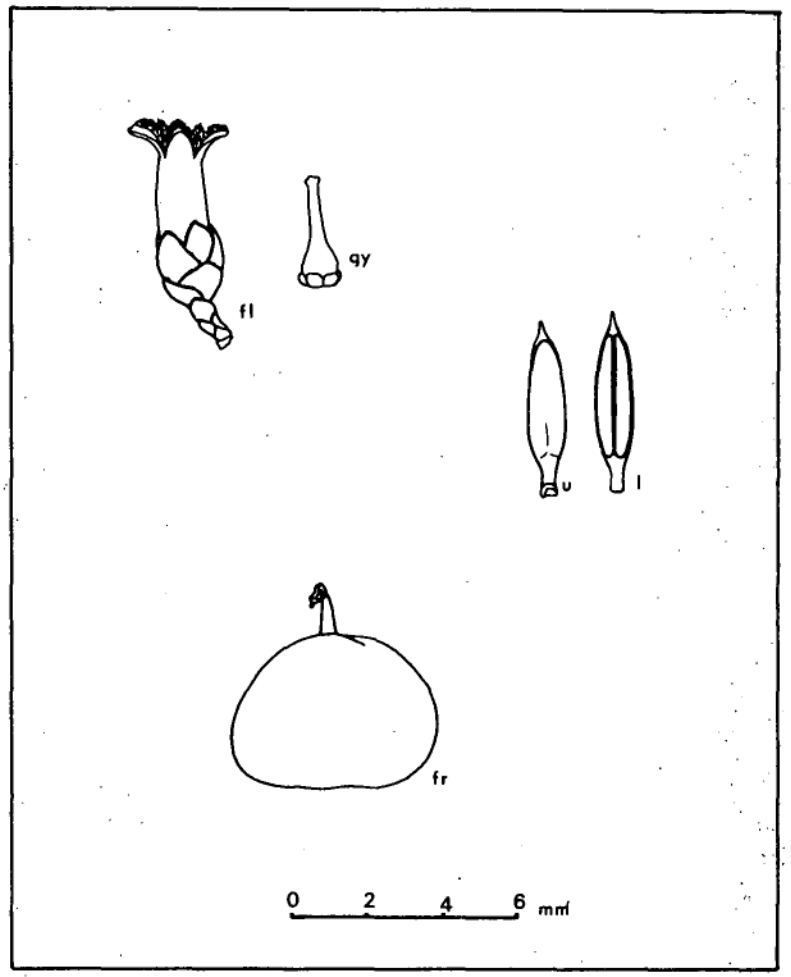


Fig. 23. *Cyathodes dealbata.*

(fl = flower
fr = fruit
gy = gynoecium
l = lower leaf surface
u = upper leaf surface)

Morphological characters

shrub size (cm.)

< 10	10 - 20	20 - 100	> 100
A	*G,H,I,J*	*B,C,D,F*	*E,K*

leaf size (cm.)

< 0.6	0.6 - 1	1 - 1.5	> 1.5
A,B,C	*G,H,I*	*D,E,J*	*F,K*

no. of veins on undersurface

1 (-3)	3 - 5	5 - 7	> 7
A	*B,C,D,E,H,I*	*F,G*	*J,K*

habit

prostrate	erect or ascending
A	*B,C,D,E,F,G,H,I,J,K*

fruit mesocarp

watery	pulpy
A	*B,C,D,E,F,G,H,I,J,K*

Chemical characters

	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*
Cy-3-XylArab	+	-	-	-	-	-	-	-	-	-	-
Cy-3-XylGal	+	-	-	-	-	-	-	-	-	-	-
Cy-3-Rha	+	+	-	-	-	+	-	-	-	-	-
Dp-3-Gal	+	-	-	-	-	-	-	-	+	-	-
dominant My glycosides	+	-	-	-	-	-	-	-	-	-	-
My-3-Rha	+	+	-	-	+	-	-	-	-	-	-
flavonol-3-glucuronides	-	+	+	+	+	+	+	+	+	+	+

Table 39. Comparison of *C.dealbata* with other Tasmanian *Cyathodes*.

(*A* = *C.dealbata*; *B* = *C.parvifolia*; *C* = *C.* var. *pendulosa*; *D* = *C.divaricata*; *E* = *C.juniperina*; *F* = *C.abietina*; *G* = *C.petiolaris*; *H* = *C.* var. *intermedia*; *I* = *C.nitida*; *J* = *C.straminea*; *K* = *C.glauca*)

C. *MONOTOCA*

Of the three genera presented in this section, *Monotoca* is the one covered least satisfactorily. A knowledge of field variation is still far from complete, and mature fruit have yet to be examined for two species, *M.scoparia* and *M.elliptica*. Chromatographic patterns are relatively complicated and the identity of several high running compounds (in BAW) remains unknown. Cross references between chromatograms of these latter pigments were not always achieved with confidence, even when they were present as dominant compounds. Hence, the genus still requires a considerable amount of work both in the field and in the laboratory before its treatment can be deemed in any way adequate.

Five species and one variety are recorded from Tasmania (Bentham - 1869, Curtis - 1903). Two species, *M.scoparia* and *M.elliptica*, also occur in New South Wales and Victoria but *M.glauca*, *M.linifolia* and *M.empetrifolia* are endemic. In addition to those species already recorded, two varieties (*M.* var. *L.Nicholls* and *M.* var. *National Park*) were also studied.

The plants vary from low shrubs less than 20 cm. high to small trees up to 6 m. They range from coastal situations to alpine habitats and are present throughout the state although particular species are restricted e.g. *M.elliptica* and *M.scoparia* have been found only in the east and north-east. The flowers are small, rarely solitary, white or cream, and usually inconspicuous. The fruits are also small (< 0.4 cm. in diameter) and fall quickly after ripening. They are usually coloured by carotenoid pigments although *M.linifolia* and *M.* var. *National Park* (*M.* var. *Nat.Pk*) have anthocyanin pigmentation. With the exception of the latter species, all show some degree of sex separation in the flowers.

Division of the genus into related groups is more difficult than in either *Epacris* or *Cyathodes*. Affinities between *M.linifolia* and *M.* var. *Nat.Pk.*, and between *M.submutica* (previously considered a variety of *M.scoparia*) and *M.* var. *L.Nicholls* (*M.* var. *L.N.*) are clear but elsewhere close relationships are not readily apparent. *M.glauca* and *M.elliptica* are similar in vegetative morphology but are distinct with respect to vegetative parts. A close similarity between *M.glauca* and *M.linifolia* has been suggested in earlier taxonomic work, since the latter was listed as a variety of the former (Rodway - 1903). However,

differences in floral and fruit characters preclude a particularly close relationship. The remaining species, *M.empetrifolia* and *M.scoparia*, appear taxonomically distinct. A discussion of the latter species with respect to *M.submutica*, is given below.

1. Comments on some species

(a) *M.* var. *L.Nicholls*

M. var. *L.N.* may be closely related to *M.rotundifolia* from Victoria, but I have not seen the latter species growing, nor have I been able to make a comparison from herbarium material. However, from the published description of *M.rotundifolia*, it differs from *M.* var. *L.N.* in having more revolute leaves, the presence of cilia at the leaf apex and a higher number of veins apparent on the leaf undersurface. The flowers are described as solitary whereas they may be solitary or arranged in short spikes of two or three flowers in *M.* var. *L.N.* As well as these differences, the corolla lobes are glabrous with flat margins in *M.* var. *L.N.* but are described as 'prominently papillose' (Willis - 1967) with narrowly incurved margins in *M.rotundifolia*.

Of the Tasmanian species, *M.* var. *L.N.* appears to be closely allied to *M.submutica* (see below) and the two are morphologically similar in many characters. Furthermore, both species show a high degree of sex separation in their breeding system, with anthers completely absent in the female plants. However, several differences also exist including habit, leaf shape and size, flowering season and number of floral bracts.

With respect to flavonoid chemistry, similarities between *M.* var. *L.N.* and *M.submutica* are relatively few (see Table 40, p. 152) and are certainly not of the order one would expect from a species and its variety. However, a comparison between *M.submutica* and its east coast form (see Table 40) suggests that there is a high degree of intraspecific chemical variation in this species. Consequently, the chemical divergence of *M.* var. *L.N.* may be less significant in this context. For the moment, however, the status of *M.* var. *L.N.* is uncertain and it seems best to consider it separately until further information comes to hand.

A description of *M.* var. *L.N.* is given on p. C10.

(b) *M.submutica*

M.submutica has previously been described as a variety of *M.scoparia* (Bentham - 1869, Rodway - 1903, Curtis - 1963). Curtis remarks -

"The status of *M.scoparia* (Sm.) R.Br. var. *submutica* Benth. needs further investigation. It is distinguished by the leaves and fruit and may prove specifically distinct."

A close comparison of morphological characters in *M.scoparia* and *M.submutica* does in fact, show that these two have very little in common (see Fig. 24, p. 151). In *M.scoparia*, the leaf apex has a fine slender point which is prickly and the leaf margins are distinctly recurved whereas in *M.submutica*, the apex has a blunt callous point and the leaf margins are flat or scarcely recurved. Also with the latter species, the actual plants are taller and more robust than those of *M.scoparia*, and the leaves are broader, with a lower length-breadth ratio. In addition, the corolla lobes are equal to, or longer, than the tube whereas the reverse situation occurs in *M.scoparia*. The fruit of *M.submutica* is usually orange-red but is occasionally yellow as recorded in *M.scoparia*. Both are dioecious or partially so, but the flowering season is separated with *M.submutica* flowering in spring (October, November) and *M.scoparia* flowering in autumn (April, May). Finally, the habitat preferences of the two species differ, with *M.submutica* occurring in wetter areas, usually associated with rainforest or subalpine forest whilst *M.scoparia* is found in relatively dry sandy heaths in the north eastern coastal areas of the state.

An examination of flavonoid chemistry shows that few compounds occur in common in the two species (see Table 40, p. 152). Of the 13 flavonols occurring in the leaves of these species, only two are accumulated in common. Myricetin derivatives are absent in *M.scoparia* but are accumulated in significant quantities in *M.submutica*. In the flowers, only 3 of the 10 compounds present are common.

From a comparison of morphological and chemical characters, it is apparent that although there are some similarities between *M.submutica* and *M.scoparia*, the differences are at least as great as those between many other species in the family and are greater than those between *Cyathodes parvifolia - C.juniperina*, *Epacris tasmanica - E.stuartii - E.barbata*, and *Leucopogon hookeri - Lissanthe montana*, all of which are maintained separately. Hence, if the principles of taxonomy are to be in any way consistent in the family, then *M.submutica* should not be maintained as a variety of *M.scoparia*. For these reasons, in this thesis, I have separated *M.submutica* from *M.scoparia* and given it species status.

A chemically distinct form of *M.submutica* was found at Coles Bay on

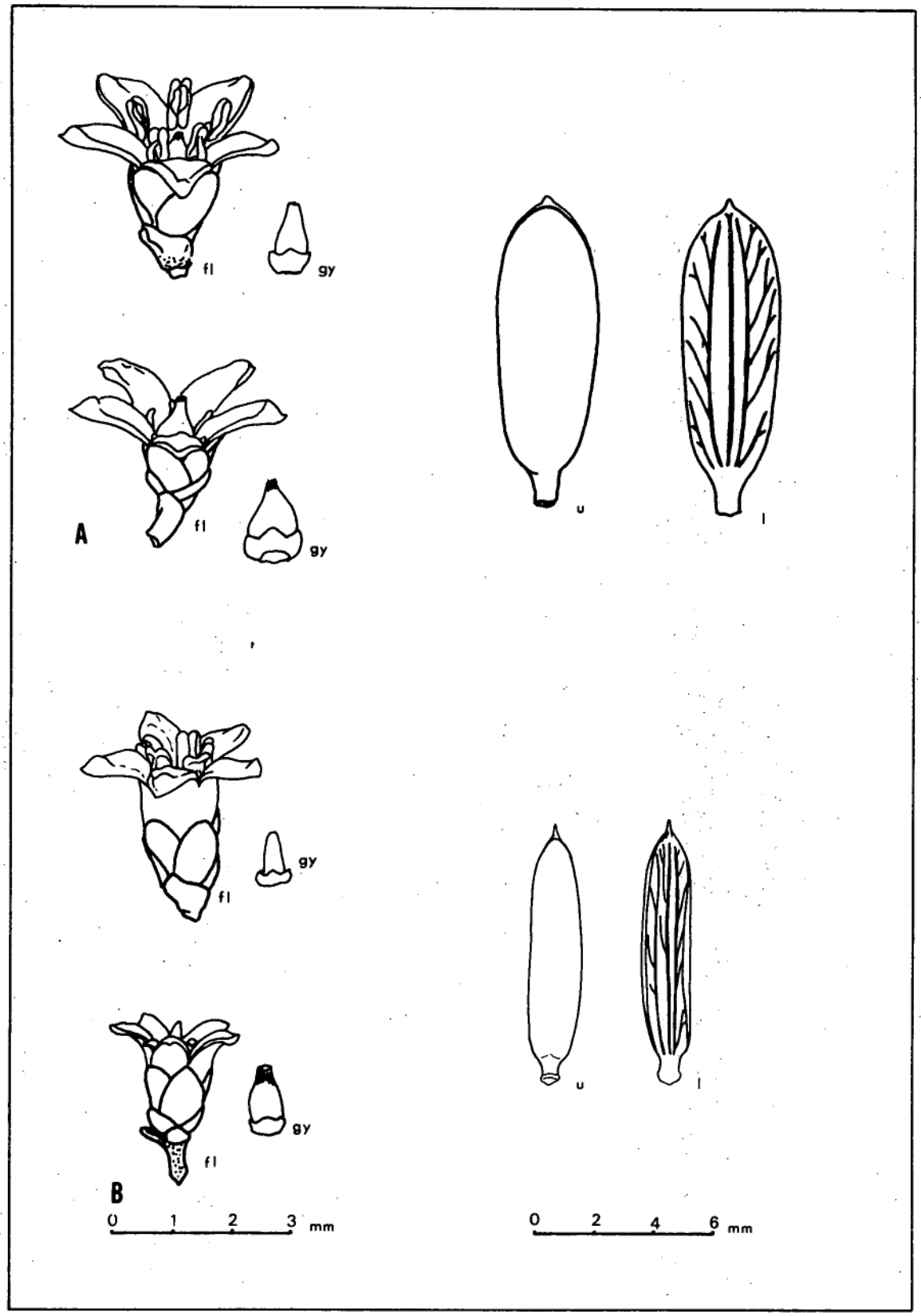


Fig. 24. *Monotoca submutica* (A) and *M.scoparia* (B). (Male and female flowers are present for both species.)

(fl = flower l = lower leaf surface
gy = gynoecium u = upper leaf surface)

the east coast. Apart from its larger leaf size, it is morphologically similar to typical *M.submutica*. A chemical comparison is shown in Table 40.

Leaves	*M.scop*	*M.sub*	*M.sub (CB)*	*M.v.L.N.*
Qu-3-Gal	+	+	+	+
Km-3-Rha	+	+	+	+
Qu-3-Glur	-	+	-	-
Qu-3-Arab	-	-	+	+
Qu-3-Rha	-	-	+	-
Km-3-Arab	-	-	+	+
My-3-Gal	-	+	+	+
My-3-Glur	-	+	-	-
My-3-Arab	-	-	+	+
Qu-3-RhaGlc	-	+	-	-
My-3-RhaGlc	-	+	-	-
D_{30}	-	-	+	+
D_{34}	+	-	+	-
D_{50}	-	-	-	+
D_{38}	+	-	-	-
D_{31}	-	+	-	+
D_{32}	-	+	-	+
D_{20}	-	-	-	+
Flowers				
Qu-3-Gal	+	+	+	+
Km-3-Rha	+	+	+	+
Qu-3-Glur	-	+	-	+
Qu-3-Arab	+	-	-	+
Qu-3-Rha	+	-	-	+
Km-3-Arab	+	-	-	-
Qu-3-RhaGlc	-	+	-	-
D_{30}	-	+	-	+
D_{34}	-	+	-	-
D_{38}	+	-	-	-
D_{31}	-	+	-	-
D_{32}	+	+	+	-

Table 40. Chemical comparison between *M.scoparia (M.scop)*, *M.submutica (M.sub)*, *M.submutica* from Coles Bay *(M.sub (CB))* and *M.* var. *L.Nicholls (M.v.L.N.)*.

(c) *M.* var. *National Park*

The species to which *M.* var. *Nat.Pk.* appears most similar is *M.linifolia* and it may be a variety of this latter species. (Comparative drawings of the two are shown in Fig. 25, p. 153).

In general, the vegetative parts of the two species are similar but *M.* var. *Nat.Pk.* usually forms a lower and more rigid shrub than *M.linifolia* with leaves which are shorter and appear thicker. These differences are not conspicuous however, and when flowering and fruiting parts

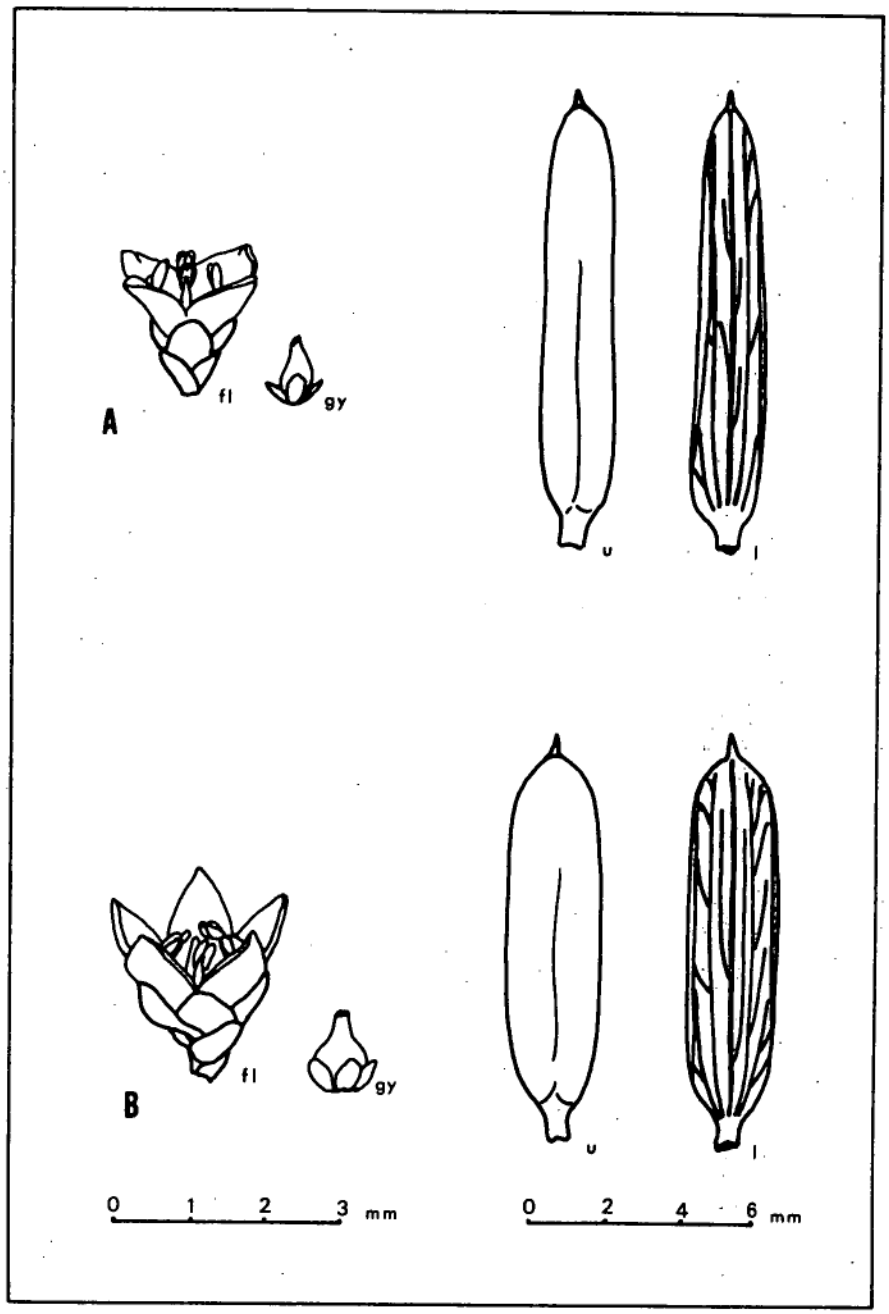


Fig. 25. *Monotoca linifolia* (A) and *M.* var. *National Park* (B).
(fl = flower l = lower leaf surface
gy = gynoecium u = upper leaf surface)

are absent, it is not always easy to distinguish the two species.

Some slight differences are apparent in fruit size and colour, with the fruit of *M.* var. *Nat.Pk.* being larger, more spherical and pinker than those of *M. linifolia.* However, these fruit characters vary greatly, even on the one plant, and overlapping forms are found. Of the two species, *M.* var. *Nat.Pk.* shows the greatest variation with respect to these characters.

The two species share a similar flowering time (October, November) and fruiting season (February - April), and their habitat preferences overlap i.e. wet heaths associated with rainforest, wet sclerophyll or subalpine forests. Of the two, *M.* var. *Nat.Pk.* tends towards the higher altitudes.

In spite of these similarities, there are also some distinct differences between *M.* var. *Nat.Pk.* and *M. linifolia.* With respect to floral characters, *M.* var. *Nat.Pk.* consistently has five sepals and petals whilst *M. linifolia* has four. There are also differences in the number of locules in the ovary. The genus is characterized by a unilocular ovary (with one exception in Western Australia) and this is true of *M. linifolia,* although some are occasionally two or three celled. In *M.* var. *Nat.Pk.*, of the 16 plants examined, 13 were characteristically bilocular, two were trilocular and only one was unilocular. In 6 of these samples, variation was apparent within plants but the overall trend for each plant was obvious. However, this examination was not undertaken as a rigorous study, and as few as 4 flowers per plant were examined for half the sample.

A comparison of pigmentation in the two indicates a close similarity in flavonoid chemistry. In all tissues examined, variation in flavonols is restricted to minor compounds. Qu- and My-3-Glur are consistently accumulated by both species, in all tissues, Km-3-Glur, Qu- and My-3-Gal in the leaves, Qu-3-RhaGlc in the flowers and Qu-3-Rha in the fruit. With respect to anthocyanins, quantitative (but not qualitative) differences are apparent in the fruit, with *M. linifolia* regularly accumulating delphinidin derivatives in excess of cyanidin derivatives whilst the converse is true for *M.* var. *Nat.Pk.* This difference is consistent with the observed difference in fruit colour.

At present, it seems that *M.* var. *Nat.Pk.* is probably a variety of *M. linifolia,* but although this is supported by chemical data, there are some distinct floral differences. Consequently, until further studies are undertaken, I prefer to consider the two separately.

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APPENDIX A

EXPERIMENTAL METHODS

A. PLANT MATERIALS

Experimental work was centred around Tasmanian plants although species from the mainland of Australia, New Zealand and South America were also included. Leaves, flowers, fruit and young twig bark were examined whenever they were available and sufficiently well developed for meaningful experimentation. Wherever possible, fresh material was used but species from New Zealand and South America could be obtained only as dried herbarium specimens. At every available opportunity, species were collected personally so that an idea of habitat, habit and morphological variation in the field could be gained. This was generally possible for Tasmanian species and for the majority of species from Victoria and New South Wales which were collected during two brief trips to the mainland. However, some species from New South Wales were obtained only as fresh cut specimens and were not seen growing in their natural environment. Voucher specimens of all species used in this study have been placed in the Botany Department Herbarium (University of Tasmania).

The collection of material in Tasmania was not always achieved without difficulty. Although the family is widespread, many species are rare or are restricted in their distribution. Many localized species are known only from areas considerably distant from the University and several of these are from the west or south-west of the state in areas which are not easily accessible. Because of these difficulties, it was not possible to obtain material in a comparable condition for all species. Even after flowering and fruiting times were established, the problem of practicability and economics remained. While species widely distant often flowered at the same time, species from the same area did not necessarily do so. Under these circumstances, even judicious planning of collecting trips was no substitute for time and money. However, of the 75 species recorded in Tasmania, only 2 *viz. Richea angustifolia* and *Sprengelia distichophylla*, were not collected at all. Both of these are rare and *S.distichophylla* is recorded only from the almost inaccessible mountains of the South-West.

B. IDENTIFICATION OF SPECIES

1. Routine Identifications

Identification of Tasmanian species was carried out according to

"The Student's Flora of Tasmania", Vol.2. (W.M.Curtis - 1963) and by comparison with specimens in the Botany Department Herbarium. For mainland species, works by Bentham (1869), Burbidge and Gray (1970), Beadle *et al.* (1972) and Willis (1972) were used. Other major works consulted for identifications included those of Brown (1810), Hooker (1860), Rodway (1903) and Allan (1961). Reference was also made to numerous regional Floras. Some species from New South Wales were already identified, or were tentatively identified so that subsequent attempts to establish their identity were greatly facilitated. A visit to the National Herbarium of New South Wales and the Herbarium Australiense in Canberra enabled identifications obtained from the literature to be confirmed.

The New Zealand species were obtained from the Botany Division of the D.S.I.R. in Cristchurch and the South American species from the National University of La Plata in Argentina. These species were identified on arrival and reference to the appropriate species descriptions indicated that there was no need to doubt the identifications.

2. Problems associated with Species Identifications

Several species were difficult to identify. Some did not fit any existing species descriptions and may represent new species or distinct but undescribed varieties of known species. Other species appeared to intergrade so that their limits were difficult to define.

Species which were particularly difficult to identify included *Cyathodes* var. *intermedia*, *C.* var. *pendulosa*, *Epacris* var. *Davies Ck.*, *E.* var. *N.S.W.*, *Leucopogon ericoides* var. *coastal*, *L.collinus* var. *alpina*, *Monotoca* var. *National Park* and *M.* var. *L.Nicholls*. Although the taxonomic status of these "species" is uncertain, they have all been named as though varieties, and are treated as separate taxonomic units even when the specific epithet has been included to show their immediate affinities. Descriptions or relevant comments for these species are given in Appendix C.

3. New Species

Two new species are described for the first time i.e. *Cyathodes nitida* and *Epacris glabella*. The description of the former species has been published (Jarman - 1974) but the second species has yet to be described in Latin (see pp. C5 and C8).

One other species listed as *Monotoca scoparia* var. *submutica* by Curtis (1963) is treated here as a distinct species i.e. *M.submutica* (see p. 149 and C9).

C. CHEMICAL ANALYSES

A list of all solvents, sprays and reagents used in the analysis of flavonoid compounds is given in Table A1b(p.A13), along with their chemical components and the abbreviations used in the text. Throughout this section, the term "flavonoid" (in inverted commas) is used as defined previously (p. 41). 1-D and 2-D represent "one dimensional" and "two dimensional" respectively. PC represents"paper chromatography".

The predominant technique used in the experimental work was that of paper chromatography. The descending method was adopted using Whatmann No.1 and 3MM paper. Standard Shandon glass tanks were used for the development of chromatograms. Other techniques included circular and column chromatography, spectral analyses, hydrolyses and limited chemical tests.

1. Extraction

For each species, material from several plants in the one population were combined for extraction and as a result, a characteristic "average" extract was obtained. With this method it was not possible to differentiate between pigments which were concentrated but present infrequently and minor constituents which were present in all the sample. Consequently, if a population is chemically variable, then the "average" extract may not give a faithful representation of individual plants. On the other hand, to choose a single plant may give an equally erroneous result. Since it is not practical to sample a large number of individuals for all populations of all species, some compromise was necessary. With"flavonoids", an average extract was obtained but at the same time, a small number of plants, usually 5 or 6, were also tested individually to gain some idea of intra-specific variation. Individual plants were usually not tested with respect to anthocyanins because the presence of these pigments was more consistent.

(a) Anthocyanins

Anthocyanins were extracted with MeOH/1% HCl until all red, pink, blue or purple colouration was lost from the tissue. When the extraction was carried out prior to column chromatography, the bulk of the extracting solvent was pure MeOH, with only a minimum of MeOH/1% HCl

present to prevent the anthocyanins from turning blue.

(b) "Flavonoids"

These were extracted with warm 70% EtOH for 4 - 12 hours.

(c) Aglycones

The mixture obtained from (b) above was boiled with 2 mls. of 1N HCl at 100°C for 45 minutes. The solution was diluted with H_2O.

(i) "Flavonoid" aglycones - the diluted aqueous solution was shaken with EtOAc and the organic layer containing the aglycones was removed and concentrated to dryness in a fast air stream.

(ii) Anthocyanidins derived from leucoanthocyanidins - the aqueous layer remaining from (i) was extracted with amyl alcohol to remove the anthocyanidins. The solution was concentrated to dryness.

Anthocyanidins derived from leucoanthocyanidins and those derived from anthocyanins are indistinguishable on paper chromatograms and for this reason analyses of leucoanthocyanidins requires some caution. Wherever possible, healthy material was chosen which showed no red, pink, purple or blue colouration. When this was not possible, either the results were carefully estimated with due regard to the anthocyanins known to be present, or else an analysis of leucoanthocyanidins was omitted.

2. Routine Analysis of Extracts

The routine analysis of extracts involved a 2-D chromatographic survey of all available Epacrid species. Anthocyanin chromatograms were viewed in visible and ultra violet (UV) light at 270 nm. and flavonol chromatograms were viewed in UV light, with and without the presence of concentrated ammonia fumes.

The routine analysis of flavonol aglycones involved both 1-D and 2-D PC. 1-D chromatograms were always run in conjunction with 2-D chromatograms because of the large number of pigments present in the crude extracts. Confident identifications using co-chromatography alone were usually impossible to achieve because colour reactions were obscured by contaminating compounds. For anthocyanidins, however, 1-D chromatography was deemed sufficient because the pigments were few in number, they were readily separated in the solvents used and could be viewed in visible light. (Contaminant pigments were usually not apparent in visible light.)

The developing solvents were -

	First Direction	Second Direction
2-D chromatograms		
Anthocyanins	BAW	5% AcOH
"Flavonoids"	BAW	H_2O or 15% AcOH
1-D chromatograms		
Anthocyanidins	Forestal, Formic Acid	
"Flavonoid" aglycones	Forestal, Formic Acid, H_2O, 15% AcOH, BAW, NH_3-Bu.	

3. Isolation and Purification of Pigments

Prior to all isolations, a 2-D chromatogram of the crude extract was run to use as a guide throughout the procedure. When the final step in the purification of a pigment involved PC, an aqueous solvent (without mineral acid) was always used. This ensured the removal of any contaminant sugars (in particular, arabinose) which may have been contributed by the paper.

(a) Anthocyanins

(i) Initial purification steps

During purification of pigments using the developing solvents BAW and 5% AcOH, considerable difficulty was frequently experienced with fruit, and occasionally flower extracts, when BAW was used as the first developing solvent. Unidentified, apparently viscous, contaminants interfered with the development of the chromatograms causing the pigment bands to become very "wavy" and often to show no separation at all. Such an effect could be partly avoided by using 5% AcOH as the first developing solvent but this was considered too inefficient to be adopted as the routine procedure because the anthocyanins usually streaked into very wide bands. To overcome these problems, two methods were investigated. Method 1 had limited success but it is included because it proved helpful in the absence of column chromatography (Method 2.).

Method 1. - The extracted solution was concentrated by rotary evaporation and streaked onto Whatman 3MM paper. The chromatogram was developed in EtOAc/(MeOH/1% HCl) from 1 - 4 hours. The anthocyanins remained at the origin but many non flavonoid contaminants including chlorophyll migrated down the paper. The anthocyanin mixture was then cut out, eluted with MAW and purified in the usual manner (see part (ii), below).

This method showed greater application to the Myrtaceae (studied

concurrently in this laboratory by another research student) than to the Epacridaceae. However, in the Epacridaceae, it was successful with several species and was certainly preferable to using 5% AcOH as the "cleaning up" solvent.

Method 2. - The extracted anthocyanin solution was concentrated to a small volume by rotary evaporation, H_2O was added and evaporation continued until all traces of MeOH were removed. The remaining aqueous solution was further diluted with a large amount of H_2O (at least 5 times the volume of the original MeOH extract), filtered to remove H_2O insoluble materials and then passed through a column containing a weak carboxylic acid ion-exchange resin, Bio-Rex 70H$^+$ (Bio-Rad Lab.). The anthocyanin mixture was then eluted from the column with MAW and concentrated to dryness by rotary evaporation. Individual anthocyanins were then purified by PC (part (ii), below).

The amount of anthocyanin that could be concentrated onto a column such as this was dependent on the pH of the aqueous pigment solution. For this reason, a minimum quantity of MeOH/1% HCl was used during the initial extraction, the bulk of the extracting fluid being MeOH. The required amount of MeOH/1% HCl varied between species and was only added to maintain the normal red colour of the anthocyanin and prevent it from changing to the bluish tones characteristic of anthocyanins at high pH's.

This preliminary treatment, while causing no apparent breakdown of pigments was very successful in providing a bulk quantity of a "cleaned" anthocyanin mixture.

(ii) Preparative Scale Paper Chromatography

The anthocyanin solution was concentrated by rotary evaporation and streaked onto Whatman 3MM paper. The chromatogram was developed in BAW, and the appropriate pigment bands were cut out and eluted individually with MAW. The pigment solution was then concentrated, and the procedure repeated using different developing solvents until the anthocyanins were purified. The usual solvents were BAW and 5% AcOH but occasionally WAH and 3% HCl were also used.

(iii) Final purification step when contaminants persist

Some difficulty was encountered in the purification of delphinidin 3-rhamnosylgalactoside and cyanidin 3-xylosylarabinoside. The former was contaminated by a colourless compound which interfered with the acid hydrolysis, resulting in the rapid disappearance of the aglycone within 12 minutes of heating. Subsequent analysis of the sugars

was difficult to interpret. Cy-3-XylArab was contaminated with a yellow flavonoid. Contaminants with neither pigment could be separated adequately by the methods described above. However, in both cases it was possible to remove the contaminant by passing a weakly acidic, aqueous solution of the partially purified anthocyanin through a short column (2 cm. long, 1.5 cm. in diameter) of polyamide-celite. The anthocyanin was collected directly onto a column of the ion-exchange resin, Zeo-Karb 226H^+ (Permutit Co.), from where it could be eluted with MAW as a pure anthocyanin. PC monitering of the process showed that this treatment caused no alteration of the anthocyanin structure.

(b) "Flavonoids"

Three different procedures were adopted for the isolation of "flavonoids". For the routine identification of pigments, preparative scale PC was used (Method 1., below). After a collection of reference compounds of known identity had been accumulated, compounds were isolated by Method 2 (below) and identified by co-chromatography. Method 3 was used to obtain a large quantity of partially purified extract which could then be completely purified by Method 1. In this manner, minor compounds became available in quantities large enough to undergo the usual identification procedures. Method 4 was used when pigments of low R_f in BAW were inseparable in both the organic and aqueous solvents.

Method 1. - Preparative Scale PC. The general method described for anthocyanins (part (ii), p. A7) was adopted but different solvents were used. The eluting solvent was 70% EtOH while the developing solvents were BAW, H_2O, 5% AcOH and 15% AcOH. For straightforward isolations, the usual order of solvents was BAW, 15% AcOH, BAW and 5% AcOH.

Method 2. - The crude extract was treated with 2-D PC. Spots appearing pure were cut out and eluted by shaking with 70% EtOH for 1 hour. The solution was filtered and concentrated as the pure pigment.

Method 3. - The fresh extract (or the dried extract redissolved in 70% EtOH) was concentrated by rotary evaporation until all the alcohol was removed. The resulting aqueous solution was thoroughly mixed with a small quantity of polyamide and then placed on top of a large polyamide - celite column (after Mabry *et al.* - 1970), 20 cm. high and 6 cm. in diameter. The column was successively washed with H_2O, 20% MeOH, 40% MeOH, ... , MeOH. 20 ml. fractions were collected, concentrated and monitered by 1-D PC using BAW, H_2O and 15% AcOH. Appropriate fractions were combined and purified using Method 1.

Method 4. - Circular PC. A sheet of Whatman 3MM paper was folded in four and placed between two glass plates, one having a small hole at the centre. The partially purified extract was applied to the paper (through this hole), and a paper wick was attached from a small petrie dish of BAW.

(c) "Flavonoid" Aglycones

Methods 1 - 3, described above (p. A8) were used. The additional developing solvents, 30% AcOH, Forestal and Formic Acid were occasionally used for PC.

4. Identification of Pigments

For anthocyanins and their aglycones, dried pigments were redissolved in MeOH/1% HCl while "flavonoids" and their aglycones were usually redissolved in MeOH. However, a few drops of MeOH/1% HCl were sometimes required also.

(a) Co-chromatography

The unknown compound and the reference pigment were spotted separately and in combination, and were run on 1-D chromatograms. A list of reference compounds and their source is shown in Table A2 (p. A14).

The developing solvents were -

Compound	
Anthocyanins	BAW, 3% HCl, WAH
"Flavonoids"	BAW, H_2O, 15% AcOH, occasionally 5% AcOH
"Flavonoid" aglycones	BAW, 15% AcOH, Forestal, Formic Acid, occasionally H_2O and NH_3-Bu

For rigorous R_f data shown in Table A3 (p. A15), 50 µl. of pigment solution, at an OD = 1, was used.

(b) Hydrolyses

(i) Complete Acid Hydrolysis

The purified pigment was boiled with 2 mls. of 1N HCl in a water bath at 100°C for 30 mins (anthocyanins) or 30 - 45 mins (flavonols). The solution was cooled and diluted with water. Anthocyanidins were extracted with amyl alcohol and were identified by co-chromatography (1-D) against known references. The developing solvents used were Forestal and Formic Acid. "Flavonoid" aglycones were extracted with EtOAc and purified from breakdown products using PC with thick paper (see Method 1 - p. A8). A small sample was co-chromatographed (1-D) in Forestal, BAW and 15% AcOH against known references. A spectral analysis was also carried out (see p. A11).

With compounds in which glucuronic acid was thought to be involved, 3% H_2SO_4 was used in place of HCl. This appeared to improve the yield of sugar.

(ii) Partial Acid Hydrolysis

In many cases, and particularly with anthocyanins, an analysis of partial hydrolysis products was helpful in establishing the presence of a disaccharide and the probable order of the sugars. The method was not as satisfactory with the flavonols because the aglycone-sugar link was hydrolysed very quickly and the intermediate products could rarely be detected.

2 mls. of 1N HCl was added to the pigment and the solution placed in a water bath at 100°C.

Anthocyanins - Small aliquots were removed at 0, 6, 12 and 30 minutes after heating had commenced, and were chromatographed (1-D with thin paper) using the solvents BAW and 5% AcOH.

"Flavonoids" - Small aliquots were removed at 0, 1, 3, 6, 12, 30 and 45 minutes after heating had commenced, and were chromatographed (1-D with thin paper) using BAW and 15% AcOH.

(iii) Hydrogen Peroxide Hydrolysis

The method of Chandler and Harper (1962) was adopted *viz.* anthocyanins were dissolved in MeOH, and flavonols were suspended in H_2O containing 0.01 mls of 0.1N NH_3. 0.04 mls of H_2O_2 were added and after 4 hours at room temperature, a few grains of Pd catalyst was added to decompose the excess peroxide. After 20 hrs., 0.05 mls. of conc. NH_4OH were added and the solution warmed for 5 mins. in boiling water. The resultant solution was used directly on the chromatogram, for sugar analysis.

(c) Sugar Analysis

After extraction of the aglycone, the acidic aqueous solution was removed and neutralized with n,n-diN-octylmethylamine in chloroform (1:9), washed twice with pure chloroform to remove any excess alkali, placed on a watch glass and allowed to dry in a vacuum dessicator over silica gel. When dry, the sugar was redissolved in one drop of water, spotted on No.1 paper and co-chromatographed against known reference sugars. The developing solvent was BBPW. In cases where the results were doubtful, a repeat run using BAW was carried out. The chromatogram was sprayed with aniline hydrogen phthalate and heated for 5 mins. at 100°C. The sugars appeared as coloured spots on the chromatogram.

D. SPECTRAL ANALYSES

1. Anthocyanins

Purified anthocyanins were dissolved in MeOH/1% HCl and the spectra scanned between 620 and 260 nm. The presence of substitution at position 5 was tested by obtaining the ratio OD_{440}/OD_{max}. Low figures (< 20%) indicate substitution at position 5 whereas relatively higher figures (> 20%) indicate substitution at position 3. The $AlCl_3$ shift was obtained by measuring the spectra after the addition of 2 drops of reagent. Although MeOH/0.01% HCl is recommended for spectra of anthocyanins, particularly when $AlCl_3$ is used, it was not found to dissolve the pigments readily and a more concentrated solution was used. This did not influence the $AlCl_3$ spectra as indicated by the bathochromic shifts with cyanidin and delphinidin.

Anthocyanins and their aglycones were not studied routinely by spectral examination. Once the range of aglycones was established and individual ones identified, all succeeding aglycone identifications were carried out by co-chromatography.

2. "Flavonoids" and their aglycones

The procedure set out by Mabry *et al.* (1970) was adopted here i.e. the pigments were dissolved in MeOH and spectrally scanned over the range from 500 to 200 nm. The effects of various reagents were determined. These were used as recommended by Mabry *et al.* (1970) and included NaOMe, $AlCl_3$, $AlCl_3$/HCl, NaOAc and H_3BO_3.

The control used for these spectra was obtained by treating a sheet of blank paper in the same manner as the last chromatographic run for the pigment in question. The eluent was concentrated and redissolved in MeOH ready for use in the spectral comparison.

E. CHEMICAL TESTS

Chemical tests were not used routinely in the analysis of pigments. The Van/HCl test was used from time to time, to test for contamination by leucoanthocyanins or catechins, and the Shinoda test was used where the class of compound was in some doubt.

1. Vanillin/HCl (Siekel - 1962)

The chromatogram was sprayed with Van/HCl (0.5 gms. in conc.HCl/EtOH - 1:1). With this treatment, leucoanthocyanidins with phloroglucinol derived groups, and catechins, turned pink in visible light.

2. Shinoda Test (Dean - 1963)

3 cm. of Magnesium ribbon was dropped into a MeOH solution of the pigment and 6 drops of conc. HCl was added. Flavones turn yellow with this treatment whilst flavonols and dihydroflavonols turn deep red or purple.

abbreviations	chemical composition	
Extracting solvents		
MeOH/1% HCl	methanol - hydrochloric acid	99:1
70% EtOH	ethanol - water	7:3
Developing solvents		
5% AcOH	water - gl. acetic acid	19:1
15% AcOH	water - gl. acetic acid	17:3
30% AcOH	water - gl. acetic acid	7:3
BAW	n-butanol - gl.acetic acid - water	4:1:5
BBPW	n-butanol - benzene - pyridine - water	6:1:4:3
BEW	n-butanol - ethanol - water	4:1:22
EtOAc/(MeOH/1%HCl)	ethyl acetate - MeOH/1% HCl	19:1
Forestal	gl. acetic acid - conc. HCl - H_2O	30:3:10
Formic acid	formic acid - conc. HCl - H_2O	5:2:3
H_2O	distilled water	
NH_3-Bu	2N ammonia - n-butanol	1:1
WAH	water - gl. acetic acid - conc. HCl	82:15:3
3% HCl	water - conc. HCl	97:3
Eluting solvents		
MAW	methanol - acetic acid - water	85:5:10

Sprays

aniline hydrogen phthalate

- 16 gms of phthalic acid dissolved in 9.15 mls aniline 490 mls n-butanol, 490 mls ether and 20 mls water.

Reagents (see Mabry *et al.* - 1970)

$AlCl_3$	-	5 gms of anhydrous $AlCl_3$ in 100 mls MeOH
HCl (for flavonol spectra)	-	water, conc. HCl (2:1)
H_3BO_3	-	powdered H_3BO_3 or H_3BO_3 saturated methanol
NaOAc	-	anhydrous powdered sodium acetate
NaOMe	-	2.5 gms in 100 mls dry MeOH

Table A1. Solvents, Sprays and Reagents.

Reference	Source
Anthocyanins and aglycones	
Cyanidin	Cy-3-RhaGlc (see below)
Delphinidin	Leucodelphinidin from *Vicia faba* (testa)
Pelargonidin	Pel-3-RhaGlc (see below)
Cy-3-RhaGlc	*Drimys lanceolata* (fruit)
Cy-3-XylGlc	*Sambucus niger* (fruit)
Pel-3-Glc	Pel-3-RhaGlc
Pel-3-RhaGlc	*Anthurium sp.* (spathe)
Flavonols and aglycones	
Quercetin	commercial
Kaempferol	commercial
Myricetin	*Camelia sinensis* (tea leaves)
Rhamnetin	*Eucalyptus sp.*
Qu-3-Gal	*Rhododendron sp.* (leaves)
Qu-3-Arab (avicularin)	*Rhododendron sp.* (leaves)
Qu-3-Rha	commercial
Qu-3-RhaGlc	commercial
Sugars	
Rha, Glc, Gal, Arab, Xyl	commercial
Rutinose	Qu-3-RhaGlc
Sambubiose	Cy-3-XylGal

Table A2. Reference pigments (and their origin) used in the identification of Epacrid pigments.

Anthocyanin	R_f (×100) in BAW	3% HCl	WAH
Cy-3-rhamnoside	58	17	32
Cy-3-arabinoside	38	7	18
Cy-3-glucoside	31	6	17
Cy-3-galactoside	30	6	17
Cy-3-xylosylarabinoside	35	20	37
Cy-3-rhamnosylglucoside	30	16	30
Cy-3-xylosylglucoside	30	26	41
Cy-3-xylosylgalactoside	29	27	42
Cy-3-rhamnosylgalactoside	28	14	28
Dp-3-arabinoside	26	3	11
Dp-3-glucoside	21	3	10
Dp-3-galactoside	18	3	10
Dp-3-rhamnosylglucoside	21	10	21
Dp-3-rhamnosylgalactoside	17	7	19

Table A3. R_f data for the major Epacrid anthocyanins.
(Cy-3-XylGlc is included for comparison although it was not detected in the Epacridaceae)

Table A4. Distribution of anthocyanins in the Epacridaceae.

1 = Cy-3-Gal
2 = Cy-3-Arab
3 = Cy-3-Glc
4 = Cy-3-Rha
5 = Cy-3-RhaGal
6 = Cy-3-RhaGlc
7 = Cy-3-XylArab
8 = Cy-3-XylGal
9 = Dp-3-Gal
10 = Dp-3-Arab
11 = Dp-3-Glc
12 = Dp-3-RhaGal
13 = Dp-3-RhaGlc

Pigment concentration

● > ⊘ > o > +

P = the number of populations examined.

u = unknown identity

Species	P	1	2	3	4	5	6	7	8	9	10	11	12	13	Minor constituents
Subfamily Epacrideae															
Archeria comberi															
Petals	1	ø	●												
Flower galls	1	ø	●												
Leaves	1	ø	●												
A.eriocarpa															
Petals	1	ø	●			o									1 u.
Capsules	1	ø	●												
A.hirtella															
Petals	1	ø	●												
Leaves	1	ø	●												
Capsules	1	ø	●												Cy-3-RhaGal?
Bark	1	ø	●												Cy-3-RhaGal?
A.serpyllifolia															
Anthers	1	●	ø												
Inflorescence	1	●	ø							o	+				
Leaves	1	ø	●							+	o				
Bark	1	ø	●							+	o				
Dracophyllum milliganii															
Anthers	1	●	ø												
Inflorescence	1	●	ø												
Leaves	1	●	ø												
D.secundum															
Inflorescence	1	●	ø												
Leaves	1	●	ø												
Epacris acuminata															
Anthers	1	ø	●												
E.barbata															
Anthers	1	ø	●												
Inflorescence	1	ø	●							o	o				
E.brevifolia															
Leaves	1	ø	●							o	o				
Bark	1	+	o							o	●				
E.corymbiflora															
Anthers	1	ø	●												
Bark	1	ø	●												
E.exerta															
Anthers	1	ø	●												
Inflorescence	1	ø	●												
E.glabella															
Inflorescence	1	ø	●												
Leaves	1	ø	●												
Bark	1	ø	●												
E.gunnii															
Inflorescence	1	ø	●			o									
Leaves	4	ø	●							+	o				
Bark	1	ø	o							o	●				
E.heteronema															
Anthers	1	ø	●												
Calyx	1	ø	●												
Bark	1	ø	●												
Epacris impressa															
Petals	11	ø	●												Pel-3-Arab? + 1 u.
Leaves	2	ø	●								o				
Bark	4	ø	●							+	●				

Species	P	1	2	3	4	5	6	7	8	9	10	11	12	13	Minor constituents
Epacris lanuginosa															
Inflorescence	1	⊘	●												
Leaves	1	⊘	●								o				
Bark	1	⊘	●								o				
E.longiflora															
Petals	1	⊘	●												
E.marginata															
Anthers	1	⊘	●												
E.microphylla															
Petals	1	⊘	●												
Leaves	1	⊘	●												
E.myrtifolia															
Inflorescence	1	⊘	●												Dp-3-Arab + 1 u.
Leaves	1	⊘	●												
Bark	1	⊘	●												
E.obtusifolia															
Inflorescence	1	⊘	●												
Leaves	1	●	⊘												1 u.
Bark	1	⊘	●												
E.paludosa															
Petals	1	⊘	●												
Leaves	1	⊘	●							+	o				
Bark	1	o	⊘							+	●				
E.petrophila															
Leaves	1	⊘	●							o	o				
Bark	1	⊘	●							⊘	●				
E.pulchella															
Petals	1	⊘	●												
E.reclinata															
Petals	1	⊘	●												
E.rigida															
Petals	1	⊘	●												
E.serpyllifolia															
Anthers	2	⊘	●												
Inflorescence	2	o	●							+	⊘				
Leaves	1	⊘	●							+	o				
Bark	1	o	●							o	⊘				
E.stuartii															
Anthers	1	⊘	●												
Calyx	1	⊘	●							o	o				
E.tasmanica															
Inflorescence	3	o	o												
Leaves	1	o	o												
E.virgata															
Anthers	1	o	o												
Calyx	1	o	o												
Prionotes cerinthoides															
Petals	2	●	●												Cy-3-XylGal? + 1 u.
Leaves+stems	2	●	●												
Capsules	1	●	●												
Richea acerosa															
Leaves	3	●	⊘												Dp-3-Gal? + 1 u.
R.continentis															
Leaves	1	●	⊘												

Species	P	1	2	3	4	5	6	7	8	9	10	11	12	13	Minor constituents
Richea dracophylla															
Leaves	1	●	⊘												
Capsules	1	●	⊘												
R.gunnii															
Operculum	1	●	⊘												
Leaves	2	●	⊘												
R.milliganii															
Leaves	1	●	⊘												
R.pandanifolia															
Operculum	1	●	⊘						o						Cy-3-Arab?
Capsules	2	●	⊘						o						1 u.
R.procera															
Operculum	1	●	⊘												
R.scoparia															
Operculum	2	●	⊘			o									
Leaves	2	●	⊘												
Capsules	1	●	⊘												
R.sprengelioides															
Operculum	1	●	⊘												
Leaves	1	●	⊘												
Sprengelia incarnata															
Inflorescence	12	⊘	●												
Leaves	2	⊘	●												
Woollsia pungens															
Petals	2	o	●							+	⊘				Mv-3-Gal? +
Calyx	2	o	●							+	⊘				Cy-3-Rha + Mv-3-Arab?
Subfamily Styphelieae															
Acrotriche serrulata															
Inflorescence	3	⊘	●			o				+	o				Dp-3-RhaGal?+Pel-3-Arab?
Bark	1	o	●							+	⊘				Cy-3-RhaGal?
Acrotriche divaricata															
Fruit	1	●	⊘												
Astroloma humifusum															
Petals	2	⊘	●							o					
Brachyloma daphnoides															
Leaf bracts	1	●	●												
Cyathodes abietina															
Anthers	1	●	⊘												
Calyx	1	●													
Fruit	2	●	o		⊘										1 u.
C.dealbata															
Inflorescence	1	●	⊘												
Fruit	5	●	o					+	⊘	o					Cy-3-Rha+Dp-3-XylGal?
C.divaricata															
Petals	1	●	⊘												
Leaves	1	●	⊘												
Fruit	2	●	⊘												
Bark	1	●	⊘												Dp-3-Gal?

Species	P	1	2	3	4	5	6	7	8	9	10	11	12	13	Minor consitiuents
Cyathodes glauca															
Leaves	5	●	o			⊘				+			+		
Fruit	6	+	+			⊘				o			●		Dp-3-Arab + 2 u.
Bark	1	●	o			+				⊘			+		
C.juniperina															
Anthers	1	●				⊘									
Leaves	1	●	⊘			o									
Fruit	7	⊘	o			●									Dp-3-Gal?+Cy-3-Rha?+1u.
Bark	1	●	⊘			o									Dp-3-Gal?
C.nitida															
Leave	1	●								⊘					
Fruit	1	●	+			o				⊘					Pe1-3-Gal?
Bark	1	●	o							⊘					
C.parvifolia															
Inflorescence	2	●	⊘			o									
Leaves	1	●	⊘												Cy-3-RhaGal?
Leaves+stems	1	●	⊘												
Fruit	6	●	⊘		+	o									Dp-3-Gal? + 1 u.
C.petiolaris															
Anthers	1	⊘	●												
Leaves	1	⊘	●												
Fruit	2	⊘	●			o					+				Dp-3-Gal?+Pe1-3-Arab?+1u.
C.straminea															
Fruit	2	o	⊘			●				+			+		Cy-3-Rha + 1 u.
C. var. *intermedia*															
Fruit	1	⊘	●			o				+	+				
C. var. *pendulosa*															
Fruit	1	●	⊘												
Leucopogon australis															
Inflorescence	4	●	⊘			o									
L.collinus															
Inflorescence	3	⊘	●												1 u.
Leaves	2	⊘	●												
Bark	2	⊘	●												
L.collinus var. *alpina*															
Petals	1	⊘	●												
Leaves	1	⊘	●												
Bark	1	⊘	●			o									
L.ericoides															
Inflorescence	2	⊘	●												Dp-3-Gal? + Dp-3-Arab?
Leaves	2	⊘	●												
Bark	2	o	●							+	⊘				
L.ericoides var. *coastal*															
Fruit	1	⊘	●												
Bark	1	⊘	●							+	●				
L.hookeri															
Inflorescence	4	●	⊘												1 u.
Leaves	2	●	⊘												
Fruit	1	●	⊘							o					
L.lanceolatus															
Inflorescence	1	●	⊘												

Species	P	1	2	3	4	5	6	7	8	9	10	11	12	13	Minor constituents
Leucopogon microphyllus															
Inflorescence	1	⊘	●												
L.milliganii															
Fruit	1	●	⊘			⊘									
L.parviflorus															
Inflorescence	1	●	⊘												
Bark	1	●	⊘			o									
L.setiger															
Anthers	1	⊘	●												
Inflorescence	1	⊘	●												
L.stuartii															
Inflorescence	3	⊘	●												
L.virgatus															
Inflorescence	25	⊘	●					o							
Leaves	1	⊘	●												
Bark	1	⊘	●												
Lissanthe montana															
Anthers	2	●	⊘												
Inflorescence	2	●	⊘												1 u.
Fruit	3	●	⊘												1 u.
L.sapida															
Fruit	1	●	⊘												
L.strigosa															
Inflorescence	11	●	⊘												Pel-3-Gal?+Pel-3-RhaGal?
Fruit	2	●	⊘												
Monotoca empetrifolia															
Inflorescence	1	●	⊘												Dp-3-Gal? + Cy-3-RhaGal?
Leaves+stems	1	●	⊘												Dp-3-Gal?
M.glauca															
Leaves	1	●	⊘												
Bark	1	●	●							⊘	⊘				
M.linifolia															
Fruit	2	⊘	+			⊘				●	o		●		
M. var. *National Park*															
Inflorescence	1	●	⊘												
Leaves	1	●	o			⊘				+					
Fruit	2	⊘	+			●				+			o		
Pentachondra ericaefolia															
Leaves+stems	1	⊘	●							o	⊘				1 u.
P.involucrata															
Petals	2	o	●			⊘									
Leaves+stems	1	●	●			⊘				o	o				
P.pumila															
Fruit	4	⊘	●			o									
Styphelia adscendens															
Calyx	1	⊘	●												Cy-3-RhaGal?
S.tubiflora															
Petals	1	⊘	●												
Leaves	1	⊘	●												

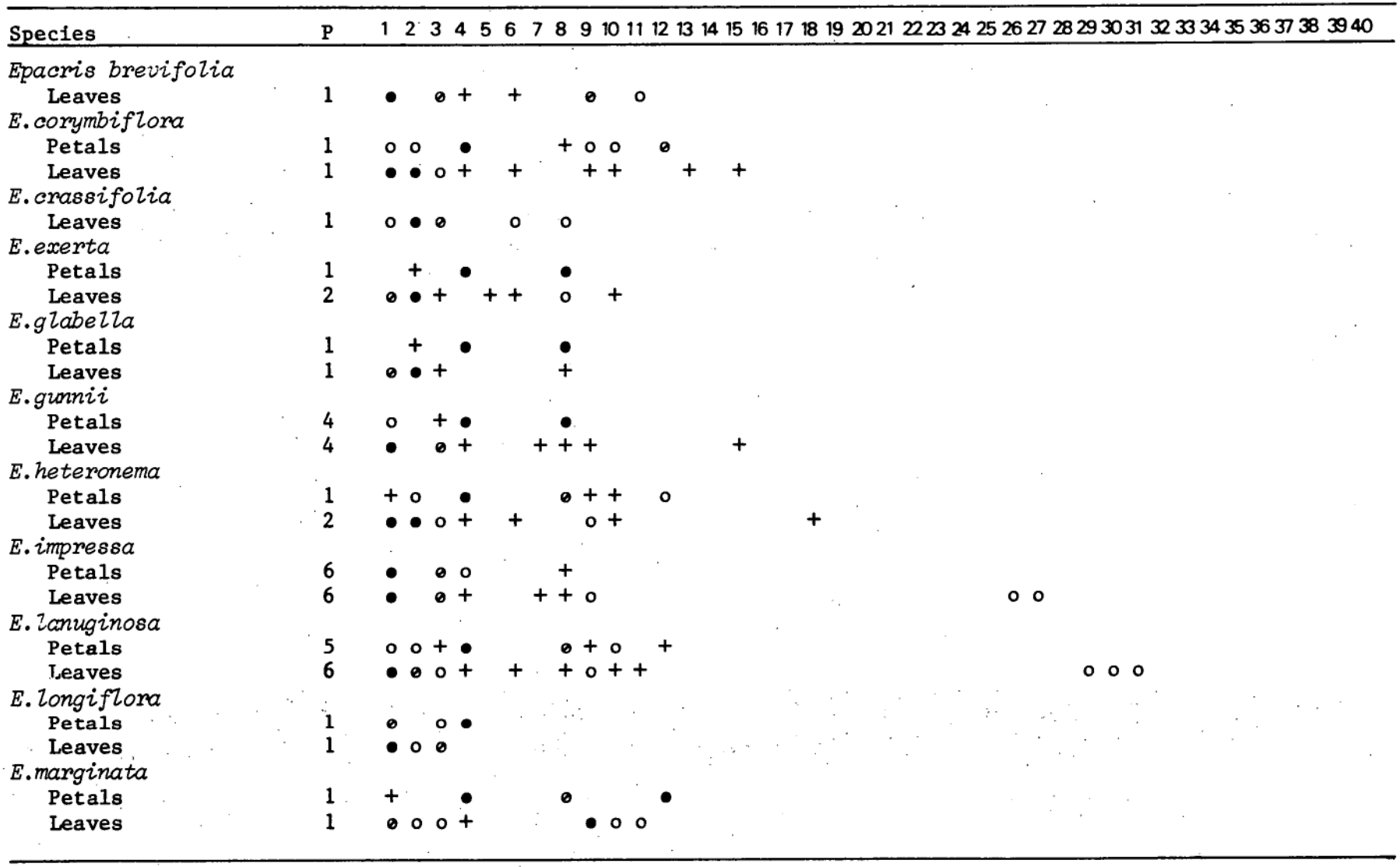
Species	P	1	2	3	4	5	6	7	8	9	10	11	12	13	Minor constituents
Trochocarpa cunninghamii															
Petals	3	●	o			⌀									
Inflorescence	3	●	o			⌀									
Leaves	2	●	o			⌀				⌀					
Fruit	5	o				⌀				+			●		Cy-3-Arab + 1 u.
T.disticha															
Petals	1			⌀			●								
Leaves	1			●			⌀								
Fruit	2			+			⌀					o		●	2 u.
T.gunnii															
Inflorescence	1			⌀			●								
Leaves	1			●			●								
Fruit	3			o			⌀					+		●	Pel-3-RhaGlc + 2u.
Bark	2			⌀			●					+		o	
T.thymifolia															
Inflorescence	1	●	+			⌀				o					
Leaves	2	●	o							⌀					
Fruit	1					⌀							●		Cy-3-Arab+Cy-&Dp-3-Gal+1u.
Bark	1	●	o							⌀					
ERICACEAE															
Gaultheria depressa															
Fruit	1	●	●												
G.hispida															
Inflorescence	1	⌀	●												
Fruit	1	⌀	●												
Pernettya tasmanica															
Fruit	2	⌀	●												

Table A5. Distribution of flavonols in the Epacridaceae.
(Pigment concentration: ● > ⊛ > o > +)

1 = Qu-3-Gal	11 = My-3-Arab	21 = D_{18}	31 = D_{44}
2 = Qu-3-Glur	12 = My-3-Rha	22 = D_{39}	32 = D_{47}
3 = Qu-3-Arab	13 = Qu-3-RhaGlc	23 = D_{31}	33 = Qu-5-Glc
4 = Qu-3-Rha	14 = Km-3-RhaGlc	24 = D_{32}	34 = Km-5-Glc
5 = Km-3-Gal	15 = My-3-RhaGlc	25 = D_{20}	35 = Pc/Black
6 = Km-3-Glur	16 = Qu-3-XylRha	26 = OPi_1	36 = D_{26a}
7 = Km-3-Arab	17 = D_{30}	27 = OPi_2	37 = D_{26b}
8 = Km-3-Rha	18 = D_{34}	28 = D_{37}	38 = Br_1
9 = My-3-Gal	19 = D_{50}	29 = D_{42}	39 = Br_2
10 = My-3-Glur	20 = D_{38}	30 = D_{43}	40 = Ch_1

P = the number of populations examined.

Species	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Subfamily Epacrideae																																									
Archeria comberi																																								
Petals	2	⊘	●	o	+		o	o	o																															
Leaves	2	⊘	●	o				o																																
A.eriocarpa																																								
Petals	2	o	●	+	o		o	+	+					⊘	+																										
Leaves	2	⊘	●	o				+						o																										
A.hirtella																																								
Petals	1	o		+	⊘			o	⊘					●	⊘																										
Leaves	2	+												●	⊘																										
A.serpyllifolia																																								
Petals	1	⊘		o				+	●					o	+																										
Leaves	1	⊘	●	+																																				
A.traversii																																								
Leaves	2	●		⊘	⊘				o						+																										
Dracophyllum milliganii																																								
Leaves	1									●		+																												
D.minimum																																								
Petals	1	o		o	●				⊘																															
Leaves	1	●	⊘																																					
D.recurvum																																								
Leaves	1	●	⊘	o	+			+																																
D.strictum																																								
Leaves	1	●	o	⊘	o		+	o	o											+		+																		
D.uniflorum																																								
Leaves	1	⊘	+	o	+					●	o	o																												
Epacris acuminata																																								
Petals	1	+	o	+	●			⊘	⊘																															
Leaves	1	●	⊘	⊘		o	o																																	
E.barbata																																								
Petals	1		+		●				●																															
Leaves	1	⊘	●	o		+	o			o	o	+																												


Species P 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
Epacris brevifolia
Leaves 1
E.corymbiflora
Petals 1
Leaves 1
E.crassifolia
Leaves 1
E.exerta
Petals 1
Leaves 2
E.glabella
Petals 1
Leaves 1
E.gunnii
Petals 4
Leaves 4
E.heteronema
Petals 1
Leaves 2
E.impressa
Petals 6
Leaves 6
E.lanuginosa
Petals 5
Leaves 6
E.longiflora
Petals 1
Leaves 1
E.marginata
Petals 1
Leaves 1

Species	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Epacris microphyllus																																									
Petals	1	+			⊘				●																																
Leaves	2	●		⊘	+					o	o			+																										
E.mucronulata																																								
Petals	1		o	+	●				⊘		+								+																				
Leaves	2	●	●	o	+		+			⊘	⊘	+						+	+																				
E.myrtifolia																																								
Petals	2	o	o		●				⊘				+						⊘										o										
Leaves	2	o	o	o	+		+			o	o	+																											
E.obtusifolia																																								
Petals	4	●		⊘	o			⊘	o																														
Leaves	5	●		⊘	⊘			o	+																														
E.paludosa																																								
Petals	1	o	o	+	●				⊘																														
Leaves	1	●	●	o	+		+		+	+	+			+																									
E.pauciflora																																								
Leaves	2	o	●	+		+	o																																
E.petrophila																																								
Leaves	1	●		⊘	o				o	o	+	o																											
E.pulchella																																								
Petals	1	o		o	●				⊘																														
Leaves	1	●		⊘	⊘																					o	o												
E.reclinata																																								
Petals	1	●	⊘	⊘	+		o		o																														
Leaves	1	●		o	o				o																	o	o												
E.rigida																																								
Leaves	1	o	●	+	⊘		o																																
E.serpyllifolia																																								
Petals	4	o	o		●				⊘	+	o		+																										
Leaves	3	●	●	o		+	+			o	o	+				+													o									
E.stuartii																																								
Petals	1		+		●				●																														
Leaves	1	⊘	●	o		o	o																																

Species	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Epacris tasmanica																																									
Petals	5		+		●				●																																
Leaves	5	⊘	●	o		+	+			+	+	+																													
E.virgata																																									
Petals	1		+		●				●																																
Leaves	1	⊘	●	+		o	o																																		
E. var. *Davies Creek*																																									
Petals	1	o	+		●			o	o	+									⊘									o													
Leaves	1	●	●	+			+		o	⊘									o	+								o				o								
E. var. *New South Wales*																																									
Leaves	1	+	●		o		⊘				o																													
Lebetanthus americanus																																									
Leaves	1	●		o	●		+	+												⊘																				
Prionotes cerinthoides																																									
Petals	2	+	●	+				+						+			⊘																							
Leaves	3	⊘	●	o			+			+	+			+		+	o																				o				
Richea acerosa																																									
Petals	1	●	⊘	o	o			o	+						o																									
Leaves	1	⊘	⊘	o	+	●		+																																
R.continentis																																									
Leaves	1	●		o	o			⊘	+																															
R.curtisii																																									
Leaves	3	●	o	⊘	o			⊘	+	+	+	+																													
R.dracophylla																																									
Petals	2	●		⊘	o			o	+																															
Leaves	2	●		⊘	o				+																															
R.gunnii																																									
Petals	3	●		o	o			⊘	+																															
Leaves	1	●		⊘	o			o	+																															
R.milliganii																																									
Petals	1	o	●	+				o		+	⊘									+	⊘																			
Leaves	1	●		o	⊘																																			

Species	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Richea pandanifolia																																										
Petals	1	●	o					⊘													+	+																			
Leaves	3	●	o	⊘	+			o	+						+																										
R.procera																																									
Petals	2	●		⊘	o			⊘	+	+																							⊘	⊘							
Leaves	2	o		⊘	●			+																																	
R.scoparia																																									
Petals	2	●		⊘	+			o													+	+																			
Leaves	4	●		o	+			⊘		o	+	+								+																				
R.sprengelioides																																									
Petals	2	●	⊘	o	o			⊘	+	o	o	+	+																												
Leaves	2	●	⊘	o	o			+		⊘	+	+								+																				
Sprengelia incarnata																																									
Sepals+Petals	4	o	●	+	●		+		⊘		+		+																												
Leaves	5	●	⊘	+	⊘			o																																
Woollsia pungens																																									
Petals	2	o		o	⊘			o	+	o	o	o	●																												
Leaves	2	⊘		o	●			o	o	o	+	o																												
Subfamily Styphelieae																																									
Acrotriche divaricata																																									
Petals	1	+		o					+					●	⊘																										
Leaves	1	o		o	+				o	+		+		●	⊘																										
A.serrulata																																									
Petals	2		●								⊘			o		+																									
Leaves	3	o	●	+	o		+		o	⊘	●	+		⊘	+	+																									
Fruit	2		●	+	+		o				o			⊘	o	+																									

Species	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Astroloma humifusum																																									
Petals	1		●																																					
Leaves	1	⊘	●		+		+		+																															
Fruit	1		●								⊘																													
A.pinifolium																																								
Petals	3																																							
Leaves	2	●		o	+												o				⊘																			
Fruit	1																																							
Brachyloma ciliatum																																								
Leaves	1	●		⊘	o																																			
B.daphnoides																																								
Petals	1	●		⊘																																				
Leaves	2	●		⊘	o				+																															
B.depressum																																								
Leaves	1	●		⊘	o				+														o																	
Cyathodes abietina																																								
Petals	2	+	o		●				⊘																															
Leaves	2	o	●		+		+		+	⊘	●	o		+																										
Fruit	2	o	●		●				o	+	⊘		o																											
C.dealbata																																								
Petals	2	+			o					●		⊘	●																											
Leaves	2	o		+	+				+	●		o	⊘																											
Fruit	2				⊘					⊘		o	●							o																				
C.divaricata																																								
Petals	2	o	●				+		+		⊘							o	o																					
Leaves	2	⊘	●	o	+		o		+	+	+	+						o	o																					
Fruit	1	o	⊘		o						●							o	o																				o	
C.empetrifolia																																								
Leaves	1	o	●		o		o			+	⊘			o		+																								
C.glauca																																								
Petals	4	+	⊘		+				+		+			●	o	+																								
Leaves	3	⊘	●	+			o		+	o	⊘			o		+																								
Fruit	3		o		+						⊘		+	●	o	o																								

Species	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Cyathodes juniperina																																									
Petals	4	+	⊘		o				o					●				o	o																	o	o				
Leaves	7	o	⊘	+	o				o	o	o	o		●				+	+																	o	o				
Fruit	4	o	⊘	+	⊘					+	+			●	o	+		o	o																	o	o		o		
C.nitida																																									
Petals	1	●	+	⊘	o				o	+																															
Leaves	1	⊘	+	o	+			+		●	+	+																													
Fruit	1	o	+	+	+					●	⊘	o																													
C.parvifolia																																									
Petals	3	+	●		o				o		⊘						o	o	o																						
Leaves	4	o	●	o	+		+		+	o	⊘	o	+	+				⊘	⊘																						
Fruit	3	+	●	+	⊘			+		+	⊘			o																									o	o	
C.petiolaris																																									
Petals	2	+	⊘		o		o		+	+	●																														
Leaves	3	●	●	o	+		+		+	⊘	⊘																														
Fruit	1	o	⊘	+	o		+			o	●	+																													
C.straminea																																									
Petals	1	+	o		⊘				o		+			●	o	+																									
Leaves	1	o	●	+	+		+		+	o	⊘	+		o	+	+																									
Fruit	2	+	⊘		o		+		+		o			●	o	+																									
C. var. *intermedia*																																									
Petals	1				+				+																															
Leaves	2	●		⊘						+																														
Fruit	1	●		⊘	o				+	o		+																												
C. var. *pendulosa*																																									
Petals	1		●		o		⊘		o					+					+																					
Leaves	1	⊘	●	o	+				+									+																						
Fruit	1		●		●		+		⊘		●																												o	
Leucopogon amplexicaulis																																									
Leaves	1			+	●																																			

Species	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Leucopogon australis																																									
Petals	2		●		●		⊘		●																															
Leaves	2	⊘	●	+	o		⊘		+																														
Fruit	2		●								+																												
L. collinus																																								
Petals	5	+	●	+	⊘		+		⊘																														
Leaves	4	o	●	o	+		⊘		+											+																			
L. collinus var. *alpina*																																								
Petals	1	+				⊘			●																														
Leaves	2	o		o	●				o	+		+	⊘	+																								o	
L. ericoides																																								
Petals	1		⊘		o		o		●		o																												
Leaves	2		●				o		+		⊘																												
L. ericoides var. *coastal*																																								
Petals	3		o		⊘		+		●		+																												
Leaves	4		●		+		o		+		⊘																												
Fruit	1		⊘		+		+	+			●																												
L. esquamatus																																								
Leaves	1	+	●	+	+		o		+																														
L. fasciculatus																																								
Leaves	1	o	●	+		o	●		+	o	⊘	+																											
L. hookeri																																								
Petals	3	⊘		o		o		+	●						+		+																						
Leaves	4	●		⊘	o			+																															
Fruit	4	●		o						⊘		o																											
L. hookeri (N.Z. i.e. *Cyathodes colensoi*)																																								
Leaves	2		●		●	o	+								+																								
L. juniperina																																								
Petals	1		●		⊘				+	+																													
Leaves	1		●		⊘	+			+																														
L. lanceolatus																																								
Leaves	1	o	●		+		o			+	o			●	o																								

Species	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Leucopogon maccraei																																									
Leaves	1	●		⊘	+				+																																
L.milliganii																																									
Leaves	2	⊘	●	o	o				+	o	⊘	+														+															
Fruit	1		●											o																											
L.parviflorus																																									
Petals	2	o	⊘		●				⊘					+																											
Leaves	3	+	●		o		o		+	+	⊘																														
Fruit	3		●		⊘		⊘																																		
L.setiger																																									
Petals	1	o	+		●				⊘																																
Leaves	1		●		+		+		+																																
L.stuartii																																									
Petals	2	⊘		o	+				●																																
Leaves	2	●		⊘	o		o	+	o																																
Fruit	2	●		o	o				+	⊘		+																													
L.stuartii (N.Z. i.e. *Cyathodes fraseri*)																																									
Leaves	1	+	●		+		o																																		
L.virgatus																																									
Petals	2		+		●				●																																
Leaves	2	⊘	●	+	+				+												o																			o	
Lissanthe montana																																									
Petals	2	⊘		+		⊘		o	●																																
Leaves	4	●		⊘	o			+	+	+					+																										
Fruit	2	●		⊘	+				+	o		+																													
L.sapida																																									
Petals	1	●		⊘	+	+		o	o																																
Leaves	1	●		⊘	o		o	o	+																																
Fruit	1	●		⊘	+			o																																	
L.strigosa																																									
Petals	2	●		⊘		o			o																																
Leaves	3	●		⊘		o			o																																
Fruit	1	●		⊘																																				

Species	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Monotoca empetrifolia																																									
Petals	2	⊘		o	o				o	●		o	o							+																				
Leaves	2	⊘		o		+				●		o																												
Fruit	2	●		⊘												+																								
M. elliptica																																								
Petals	2	o	o						●															⊘	+															
Leaves	3	⊘	●				o		o	+	⊘									+				o	o															
M. glauca																																								
Leaves	2	o	●				o		+	+	⊘																												
M. linifolia																																								
Petals	1		●						+		⊘			+																									
Leaves	3	⊘	●				o			o	⊘																												
Fruit	3		●		+						o		+																										
M. scoparia																																								
Petals	1	⊘		+	o			●	⊘							+				+					+														
Leaves	2	●					o			o									o		o																		
M. submutica																																								
Petals	2	o	⊘						o					+				o	+					●	o														
Leaves	1	⊘	●						o	o	⊘			o		+								o	o														
Fruit	3	⊘	⊘											+				●	+					o	o														
M. submutica (from Coles Bay)																																								
Petals	1	o							●																+														
Leaves	1	●		o	⊘			o	⊘	●		+						+	o			+																
M. var. *L.Nicholls*																																								
Petals	1	o	+	+	o				●									⊘																						
Leaves	2	⊘						+	⊘	●		o						o		+				o	o	o													
M. var. *National Park*																																								
Petals	2		●								⊘			+		+																							
Leaves	2	⊘	●		+		o		+	●	⊘									+																		
Fruit	2	+	●		o						o																												

Species	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Pentachondra ericaefolia																																									
Petals	1								⊘	●	⊘																														
Leaves	2		o		+				o	●	⊘																														
P.involucrata																																								
Petals	1		o	o	+				⊘					●	o																										
Leaves	1		●		⊘	+								⊘	o																										
P.pumila																																								
Petals	1		+	●		+			⊘					o																										
Leaves	2		●	⊘	o	+		o		+	o																													
Fruit	1		+	●		+			+		⊘			⊘																										
Styphelia adscendens																																								
Petals	1			●			o				⊘			+		+																								
Leaves	3			●		+	⊘				+																													
Fruit	1			⊘							●																													
S.laeta																																								
Leaves	1			●		o	o																																	
S.triflora																																								
Petals	1			●			+				⊘			+		+																								
Leaves	1			●		o	⊘																																	
S.tubiflora																																								
Leaves	1			●			⊘																																	
Trochocarpa cunninghamii																																								
Petals	1		⊘		o				●					o										+																
Leaves	2		●	●	o	+			+	⊘	⊘	+		⊘	o																										
Fruit	3										●					●																								
T.disticha																																								
Leaves	1		o	⊘	+	+			+	o	o	+		●	o	+																								
Fruit	1																																							
T.gunnii																																								
Petals	2			●		o	+		+					⊘																										
Leaves	1		⊘	●	o	o	o		+		o			o		+																								
Fruit	1			⊘							●					o																								

Species	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Trochocarpa thymifolia																																									
Petals	2		o		+				⌀	●	o			+		o																									
Leaves	2		⌀			+		+		●	o	o	+													o														
Fruit	2									⌀	●					●																									
T. laurina																																								
Leaves	1		⌀		+	●	o																																	
ERICACEAE																																								
Gaultheria depressa																																								
Leaves	1		●	●	+									⌀																											
G. hispida																																								
Petals	1			●		⌀																																		
Leaves	1		⌀	●				⌀																																
Permettya tasmanica																																								
Petals	1		●	+																																				
Leaves	1		●	⌀	+			+																																
Fruit	1		●	⌀	+																																			

Table A6. The occurrence of leucoanthocyanidins in the Epacridaceae. Cyanidin was present in all species and has not been included. The occurrence of myricetin observed from 2-D glycoside papers i.e. M (g), and from hydrolysed extracts i.e. M (h), is also given for comparison.

(LD = delphinidin derived from leucodelphinidin)
nd = not determined)

Species	LD	M (g)	M (h)
Subfamily Epacrideae			
Archeria comberi			
Leaves	-	-	-
Dracophyllum milliganii			
Leaves	-	+	nd
Epacris acuminata			
Petals	+	-	-
Leaves	+	-	-
E.barbata			
Petals	+	-	-
Leaves	+	+	+
E.brevifolia			
Leaves	+	+	+
E.corymbiflora			
Petals	-	+	-
Leaves	+	+	-
E.exerta			
Petals	-	-	-
Leaves	+	+	-
E.glabella			
Petals	-	-	-
Leaves	+	-	-
E.gunnii			
Petals	-	-	-
Leaves	+	-	-
E.heteronema			
Petals	-	+	-
Leaves	+	+	+
E.impressa			
Petals	nd	-	-
Leaves	+	-	-
E.lanuginosa			
Leaves	+	+	-
E.longiflora			
Petals	+	-	-
Leaves	+	-	-
E.marginata			
Petals	+	+	-
Leaves	+	+	+
E.microphylla			
Leaves	+	+	+
E.mucronulata			
Petals	-	+	-
Leaves	+	+	+
E.myrtifolia			
Petals	+	+	-
Leaves	+	-	-
E.obtusifolia			
Petals	+	-	-
Leaves	+	-	-
E.paludosa			
Leaves	+	+	+
E.reclinata			
Petals	+	-	-
Leaves	+	-	-

Species	LD	M (g)	M (h)
Epacris serpyllifolia			
Petals	nd	-	-
Leaves	nd	+	+
E.stuartii			
Petals	-	-	-
Leaves	+	-	-
E.tasmanica			
Petals	-	-	-
Leaves	+	+	+
E.virgata			
Petals	+	-	-
Leaves	+	-	-
E. var. *Davies Ck.*			
Petals	-	+	-
Leaves	+	+	+
E. var. *N.S.W.*			
Leaves	+	+	+
Prionotes cerinthoides			
Leaves	-	+	-
Richea continentis			
Leaves	-	+	+
R.dracophylla			
Leaves	-	-	-
R.milliganii			
Leaves	-	-	-
R.procera			
Leaves	-	-	-
R.scoparia			
Leaves	-	+	+
R.sprengelioides			
Leaves	nd	+	-
Sprengelia incarnata			
Leaves	-	-	-
Woollsia pungens			
Petals	+	+	+
Leaves	+	+	+
Subfamily Styphelieae			
Acrotriche divaricata			
Leaves	+	+	+
A.serrulata			
Petals	nd	+	-
Leaves	+	+	+
Astroloma humifusum			
Fruit	-	+	-
A.pinifolium			
Petals	+	-	-
Brachyloma ciliatum			
Leaves	+	-	-
B.daphnoides			
Leaves	+	-	-
B.depressum			
Leaves	+	-	-

Species	LD	M (g)	M (h)
Cyathodes abietina			
Petals	-	-	-
Leaves	+	+	+
Fruit	+	+	+
C.dealbata			
Petals	+	+	+
Leaves	+	+	+
C.juniperina			
Petals	-	-	-
Leaves	+	+	+
Fruit	+	+	+
C.parvifolia			
Petals	+	+	-
Leaves	+	+	+
Fruit	+	+	-
C.petiolaris			
Petals	+	+	-
Leaves	+	+	+
Leucopogon amplexicaulis			
Leaves	-	-	-
L.australis			
Petals	-	-	-
Leaves	-	-	-
L.collinus			
Petals	-	-	-
Leaves	+	-	-
L.ericoides			
Petals	-	+	-
Leaves	+	+	+
L.hookeri			
Petals	-	-	-
Leaves	+	-	-
L.juniperinus			
Petals	-	+	+
Leaves	-	-	-
L.lanceolatus			
Leaves	+	+	+
L.parviflorus			
Petals	-	-	-
Leaves	+	+	+
L.stuartii			
Petals	-	-	-
Leaves	-	-	-
L.virgatus			
Petals	+	-	-
Leaves	+	-	-
L.collinus var. *alpina*			
Petals	-	-	-
Leaves	-	+	+
Lissanthe montana			
Leaves	+	-	-
L.sapida			
Petals	-	-	-
Leaves	-	-	-

Species	LD	M (g)	M (h)
Lissanthe strigosa			
Petals	-	-	-
Leaves	+	-	-
Monotoca elliptica			
Petals	-	-	-
Leaves	+	+	+
M.empetrifolia			
Petals	+	+	+
Leaves	+	+	+
M.glauca			
Leaves	+	+	+
M.linifolia			
Leaves	+	+	-
M.scoparia			
Petals	-	-	-
Leaves	-	+	+
M.submutica			
Petals	nd	-	-
Leaves	nd	+	+
M. var. *Nat.Pk.*			
Petals	nd	+	-
Leaves	nd	+	+
Pentachondra ericaefolia			
Leaves	+	+	+
P.involucrata			
Leaves	nd	-	-
P.pumila			
Leaves	+	+	+
Styphelia adscendens			
Leaves	+	+	-
S.triflora			
Petals	-	+	+
Leaves	-	-	-
S.laeta			
Leaves	-	-	-
Trochocarpa cunninghamii			
Petals	-	-	-
Leaves	+	+	+

SPECTRAL DATA

Spectral data for the following compounds are given -

1. Quercetin
2. Quercetin 3-galactoside
3. Quercetin 3-glucuronide
4. Quercetin 3-arabinoside
5. Quercetin 3-rhamnoside
6. Quercetin 3-rhamnosylglucoside
7. Quercetin 3-xylosylrhamnoside
8. Quercetin 5-glucoside
9. Kaempferol
10. Kaempferol 3-glucuronide
11. Kaempferol 3-arabinoside
12. Kaempferol 3-rhamnoside
13. Myricetin
14. Myricetin 3-galactoside
15. Myricetin 3-glucuronide
16. Isorhamnetin(?) 3-rhamnosylglucoside
17. OPi_1 / aglycone
18. OPi_1
19. Pc/Black
20. Quercetin 3-galactoside (from *Rhododendron*)

1. Quercetin

Spectra (nm)

MeOH	254	270sh	300	370		
NaOMe	244	300sh	332	425	(decomp.)	
$AlCl_3$	254	260sh	300sh	310sh	362	442
$AlCl_3$/HCl	254	258sh	305	315sh	360	420
NaOAc	258sh	272	322	392	(decomp.)	
H_3BO_3	259	290sh	300sh	385		

Shifts

	NaOMe	$AlCl_3$	HCl	NaOAc	H_3BO_3
Band I	decomp.	72	50	decomp.	15
Band II	-10	0	0	18	5

2. Quercetin 3-galactoside

Spectra (nm)

MeOH	255	265sh	308sh	357	
NaOMe	271	327	410		
$AlCl_3$	273	300sh	338sh	434	
$AlCl_3$/HCl	267	297	360	400	
NaOAc	272	322	387		
H_3BO_3	260	296	378		

Shifts

	NaOMe	$AlCl_3$	HCl	NaOAc	H_3BO_3
Band I	53	77	43	30	21
Band II	16	18	12	17	5

3. Quercetin 3-glucuronide

Spectra (nm)

MeOH	255	264sh	310sh	359	
NaOMe	271	330sh	404		
$AlCl_3$	270	300sh	407		
$AlCl_3$/HCl	268	294	365	400	
NaOAc	270	315	342sh	404	
H_3BO_3	261	296	305sh	377	

Shifts

	NaOMe	$AlCl_3$	HCl	NaOAc	H_3BO_3
Band I	45	48	41	45	18
Band II	16	15	13	15	6

4. Quercetin 3-arabinoside

Spectra (nm)

MeOH	255	270sh	308sh	358	
NaOMe	271	322	410		
$AlCl_3$	255sh	273	305sh	370sh	425
$AlCl_3$/HCl	255sh	261	300	362	395sh
NaOAc	273	323	387		
H_3BO_3	261	308sh	376		

Shifts

	NaOMe	$AlCl_3$	HCl	NaOAc	H_3BO_3
Band I	52	67	37	29	18
Band II	16	18	6	18	6

5. Quercetin 3-rhamnoside

Spectra (nm)

MeOH	256	264sh	308sh	350	
NaOMe	269	325	394		
$AlCl_3$	272	290sh	329sh	425	
$AlCl_3$/HCl	269	295	354	395	
NaOAc	270	326sh	376		
H_3BO_3	258	310sh	367		

Shifts

	NaOMe	$AlCl_3$	HCl	NaOAc	H_3BO_3
Band I	44	75	45	26	17
Band II	13	16	13	14	2

6. Quercetin 3-rhamnosylglucoside

Spectra (nm)

MeOH	254	270sh	310sh	357	
NaOMe	270	321	411		
$AlCl_3$	273	298sh	416		
$AlCl_3$/HCl	270	300sh	362	399	
NaOAc	272	320	398		
H_3BO_3	260	290	370		

Shifts

	NaOMe	$AlCl_3$	HCl	NaOAc	H_3BO_3
Band I	55	59	43	42	13
Band II	16	19	16	18	16

7. Quercetin 3-xylosylrhamnoside

Spectra (nm)

MeOH	255	264sh	347				
NaOMe	270	320	401				
$AlCl_3$	255	261	274	315sh	370sh	422	
$AlCl_3$/HCl	255	261sh	270sh	304sh	310sh	358	384sh
NaOAc	270	323sh	397				
H_3BO_3	259	300sh	310sh	367			

Shifts

	NaOMe	$AlCl_3$	HCl	NaOAc	H_3BO_3
Band I	52	73	9(35?)	48	18
Band II	16	19	0	15	4

8. Quercetin 5-glucoside

Spectra (nm)

MeOH	250	270sh	280sh	368		
NaOMe	242	295	329	450	(rapid decomp.)	
$AlCl_3$	262sh	270sh	300sh	310sh	363	435
$AlCl_3$/HCl	261	270sh	305sh	363	425	
NaOAc	277	285sh	329	445	(slow decomp.)	
H_3BO_3	250sh	272	280sh	335sh	385	

Shifts

	NaOMe	$AlCl_3$	HCl	NaOAc	H_3BO_3
Band I	82	67	57	77	17
Band II	45	12	11	27	22

9. Kaempferol

Spectra (nm)

MeOH	250sh	266	291	318sh	365		
NaOMe	285sh	290	324	442	(decomp.)		
$AlCl_3$	254	260sh	268sh	305	320sh	353	420
$AlCl_3$/HCl	254	259sh	265sh	303	320sh	353	418
NaOAc	274	320	386	(very slow decomp.)			
H_3BO_3	266	292	318sh	366			

Shifts

	NaOMe	$AlCl_3$	HCl	NaOAc	H_3BO_3
Band I	77	55	53	21	1
Band II	24	-12	-12	8	0

13. Myricetin

Spectra (nm)

MeOH	247sh	295sh	373		
NaOMe	269sh	278sh	318	(rapid decomp.)	
$AlCl_3$	269	314	375sh	450	
$AlCl_3$/HCl	264	269sh	309	364	429
NaOAc	- not obtained	(The same information was available from			
H_3BO_3	- not obtained	the glycoside spectra.)			

Shifts

	NaOMe	$AlCl_3$	HCl
Band I	decomp.	77	56
Band II	?	?	?

14. Myricetin 3-galactoside

Spectra (nm)

MeOH	255sh	262	310sh	364
NaOMe	250sh	265sh	310sh	396
$AlCl_3$	272	315	430	
$AlCl_3$/HCl	273	310	375sh	406
NaOAc	272	322	398	
H_3BO_3	257	290sh	383	

Shifts

	NaOMe	$AlCl_3$	HCl	NaOAc	H_3BO_3
Band I	32	66	32	34	19
Band II	?	10	11	10	-5

15. Myricetin 3-glucuronide

Spectra (nm)

MeOH	255	265sh	302	363
NaOMe	271	320	404	(slow decomp.)
$AlCl_3$	269	310sh	412	
$AlCl_3$/HCl	270	308	368	403
NaOAc	271	318	387	(very slow decomp.)
H_3BO_3	260	305	385	

Shifts

	NaOMe	$AlCl_3$	HCl	NaOAc	H_3BO_3
Band I	41	49	40	24	22
Band II	16	14	15	16	5

16. Isorhamnetin(?) 3-rhamnosylglucoside

Spectra (nm)

MeOH	255	268	300sh	310sh	356
NaOMe	273	332	415		
$AlCl_3$	274	303	368sh	403	
$AlCl_3$/HCl	274	303sh	360	400	
NaOAc	274	322sh	402		
H_3BO_3	258sh	268	305sh	361	

Shifts

	NaOMe	$AlCl_3$	HCl	NaOAc	H_3BO_3
Band I	59	47	44	46	5
Band II	5	6	6	6	0

17. OPi1 / aglycone

Spectra (nm)

MeOH	285	341			
NaOMe	233	286	333sh	388	460
$AlCl_3$	310	364			
$AlCl_3$/HCl	310	365			
NaOAc	287	330			
H_3BO_3	289	335			

Shifts

	NaOMe	$AlCl_3$	HCl	NaOAc	H_3BO_3
Band I	97(?)	26	26	-8	-3
Band II	42	24	24	1	3

18. OPi1

Spectra (nm)

MeOH	285	341				
NaOMe	233	286	333sh	388	460	(increase → chalcone)
$AlCl_3$	221	308	395			
$AlCl_3$/HCl	220	308	395			
NaOAc	286	343				
H_3BO_3	286	343				

Shifts

	NaOMe	$AlCl_3$	HCl	NaOAc	H_3BO_3
Band I	121	54	54	2	2
Band II	1	23	23	1	1

19. Pc/Black

Spectra (nm)

MeOH	274	329			
NaOMe	252sh	273	292sh	383	
$AlCl_3$	260	284sh	294sh	300	354
$AlCl_3$/HCl	256	290sh	300	349	
NaOAc	273	296	335	372sh	
H_3BO_3	273	333			

Shifts

	NaOMe	$AlCl_3$	HCl	NaOAc	H_3BO_3
Band I	54	25	20	6	4
Band II	-1	27	27	0	0

20. Quercetin 3-galactoside (from *Rhodendron sp.*)

Spectra (nm)

MeOH	255	268sh	312sh	358	
NaOMe	272	325	411		
$AlCl_3$	273	300sh	330sh	430	
$AlCl_3$/HCl	266	272sh	300	365	397
NaOAc	269	415			
H_3BO_3	263	300sh	385	420sh	

Shifts

	NaOMe	$AlCl_3$	HCl	NaOAc	H_3BO_3
Band I	53	72	39	57	27
Band II	17	18	11	14	8

APPENDIX B

NUMERICAL PROCEDURES

Details of programmes used in the numerical analyses are given below. These programmes are held in Canberra, from where they are available to users of the Cyber 76 computer, via the C.S.I.R.O. computer network.

In this discussion, the procedures are considered in the following order - similarity measures (p. B2) and clustering strategies (p. B3) used in the different programmes and finally, the general approach employed by each programme (p. B5).

1. Similarity Measures (Sokal and Sneath - 1963)

For the first 3 coefficients, the following symbolism will be used -

D = coefficient of dissimilarity
S = coefficient of similarity
a+b+c+d = total number of characters considered

where a = the number of characters common to 2 taxa
d = the number of characters absent from 2 taxa
b and c = the number of characters present in one taxon and not in the other, and *vice versa*.

In all the programmes described here, the similarity coefficient was used to derive a dissimilarity measure such that

$$D = 1 - S.$$

(a) Simple Matching Coefficient (S_{SM})

Dissimilarity coefficients based on S_{SM} were generated in MULTBET when binary data (but not ordered multi-state data) were considered. It was not used in the formation of the classification but was used in the derivation of the principal co-ordinates (GOWER).

$$S_{SM} = \frac{a+d}{a+b+c+d}$$

With the simple matching coefficient, the existence of joint absences in two taxa is considered representative of similarity and is weighted equally with joint presence.

(b) Jaccard Coefficient (S_J)

The Jaccard coefficient was used in the classificatory programme, CLASS, and the ordination programme, GOWER.

$$S_J = \frac{a}{a+b+c}$$

Joint absences are excluded from the calculation i.e. they are not used as a measure of similarity.

(c) Kulczynski Coefficient (S_K)

This coefficient was used in CLASS and GOWER.

$$S_K = \frac{a}{2}\left(\frac{1}{a+b} + \frac{1}{a+c}\right)$$

The possible contribution of a single taxon to the similarity measure is taken as 1. The actual contribution is given by the ratio of the number of attributes held in common with the second taxon (a) to the total number of attributes present in the first taxon (a+b) i.e. a/(a+b). The values for both taxa are summed and the ratio of this value to the total possible contribution of both species together (2) is given as the measure of similarity. Joint absences are not included in this coefficient.

(d) Canberra Metric (Lance and Williams - 1967b)

The Canberra metric is used in REMUL to calculate the similarity between elements and groups while testing for misclassification of individuals. It is based on the formula -

$$S = \frac{(|x_{ih} - x_{jh}|)}{x_{ih} + x_{jh}}$$

where x_{ih} is the number of individuals in group i having the h^{th} attribute.

2. Clustering Strategies

(a) Information Statistic (Lance and Williams - 1967b)

The information statistic is used as the sorting strategy for MULTBET, DIVINF and apparently for REMUL. It is an intensely clustering strategy.

For binary data, the information content (I) for a group of individuals is calculated by the formula

$$I = pn\ln n - \{x_h \ln x_h - (n - x_h)\ln(n - x_h)\}$$

where p = the number of attributes, n = the number of individuals and x_h = the number of individuals possessing the h^{th} attribute.

The information change (Δ_I) between two groups or elements is calculated thus -

$$\Delta_I = I_{A+B} - I_A - I_B$$

where A and B are the elements of groups under consideration.

Where groups A and B consist of single individuals only, the information contribution of each attribute is either zero (when double matches occur) or $2\ln 2$ when differences occur. Hence, the total Δ_I between the two individuals would be $2p\ln 2$.

With ordered multi-state data, for the two groups involved in fusion, a set of (m - 1) 2×2 contingency tables are constructed for each individual attribute having m states. The Δ_I is calculated for each contingency table and the largest value is selected to represent the contribution of information for this attribute towards the fusion of the two groups. The (m - 1) contingency tables are obtained by dividing dichotomously at each attribute and accumulating the frequencies on either side of the division i.e.

attribute state

	1	2	3	m
Gp.A	x_{1A}	x_{2A}	x_{3A}	x_{mA}
Gp.B	x_{1B}	x_{2B}	x_{3B}	x_{mB}

→

x_{1A}	$x_{2A}+x_{3A}+.....x_{mA}$
x_{1B}	$x_{2B}+x_{3B}+.....x_{mB}$

;

$x_{1A}+x_{2A}$	$x_{3A}+.....x_{mA}$
$x_{1B}+x_{2B}$	$x_{3B}+.....x_{mB}$

; ..... ;

$x_{1A}+x_{2A}+....x_{(m-1)A}$	x_{mA}
$x_{1A}+x_{2B}+....x_{(m-1)B}$	x_{mB}

The final Δ_I resulting from the fusion of the two groups is obtained by summing the Δ_{Imax} for each individual attribute.

As with binary data, when the two groups consist of a single species each, the information contribution of every attribute towards the fusion of the groups is either zero (for double matches) or $2\ln 2$ (for differences).

(b) Incremental Sum of Squares (Burr - 1970)

This is an intensely clustering, combinatorial strategy available

for use in the programme CLASS. A dissimilarity matrix is constructed (based on S_J and S_K in these analyses) and fusion occurs between the two lowest values. The new inter-element values are calculated using the formula -

$$d_{i(j,k)} = \frac{x_i+x_j}{x_i+x_j+x_k} \cdot d_{ij} + \frac{x_i+x_k}{x_i+x_j+x_k} \cdot d_{ik} - \frac{x_i}{x_i+x_j+x_k} \cdot d_{jk}$$

where $d_{i(j,k)}$ is the distance between group (or individual) i and the new group (j,k), and x_j is the number of individuals in group j.

(c) Group Average (Burr - 1970)

This is a weakly clustering, combinatorial strategy, available for use in CLASS. New inter-element values are based on the formula -

$$d_{i(j,k)} = \frac{x_j}{x_{jk}} \cdot d_{ij} + \frac{x_k}{x_{jk}} \cdot d_{ik}$$

where $d_{i(j,k)}$ is the distance between group (or individual) i and the new group (j,k), and x_j is the number of individuals in group j.

3. Classificatory Programmes

(a) MULTBET (Lance and Williams - 1967b)

MULTBET is based on an agglomerative system and is polythetic. It is non combinatorial and uses the intensely clustering information statistic as the sorting strategy.

The information content of every pair of groups (or elements - in the first fusions) is calculated before and after fusion, and that fusion involving the smallest change in information content is selected for use in the classification (see pp. B3 and B4). The process is repeated until all elements or groups have been fused into a single large group.

A wide range of data types is accepted by MULTBET but only qualitative (binary) and ordered multi-state were applicable to these studies. When qualitative data is used, a dissimilarity matrix based on S_{SM} (see p. B2) is derived. These values were not used as such in MULTBET but were stored ready for use in the ordination analysis which was programmed to follow.

(b) CLASS (Lance and Williams - 1967a)

CLASS is based on an agglomerative system and is polythetic. It is combinatorial, with the choice of dissimilarity measure being specified by the user. Several clustering strategies are also available. In this work, only two were used i.e. the incremental sum of squares (p. B4-5), and group average (above).

As with MULTBET, an ordination analysis may be programmed to follow CLASS, and the dissimilarity values already generated are used.

(c) DIVINF (Lance and Williams - 1968)

This programme is based on a divisive strategy and is monothetic. It is non combinatorial and uses the information statistic as the clustering strategy. DIVINF accepts only qualitative data.

The information content for the total data is calculated using the information statistic (pp. B3-4). The species are then arranged into two groups depending on the presence or absence of a particular attribute. The information content of the two new groups is calculated and the difference between the sum of these and the information content obtained for the original group is found. The process is repeated on the original group using every attribute in turn as the basis of subdivision into two groups. The resulting Δ_Is are compared and that attribute (and its consequent subdivision) is selected which provides the largest information change (i.e. the two groups are internally more homogeneous than any other two groups, and are therefore more dissimilar to each other). The process is repeated for the two subgroups, and continues until the classification is completed or is terminated at the specified number of groups. The attribute on which the division is based is mutually exclusive in the two resulting groups and cannot be used again in subsequent divisions.

The classification resulting from this procedure is considered "normal" i.e. it is a classification of individuals characterized by the possession of attributes. It is also possible with DIVINF to obtain an "inverse" classification i.e. the attributes are classified as taxonomic units. From the "normal" and "inverse" classifications obtained from the same set of data, a contingency table may be constructed which indicates how groups of species may be defined by groups of attributes and *vice versa*.

(d) REMUL (unpublished)

The programme REMUL has not been published and only limited information is available for it (personal communication between Dr. Williams - Canberra - and Dr. Ratkowsky - Hobart). It is based on a divisive system and is polythetic. It permits re-allocation of misclassified species at any stage throughout the computation. By comparison with DIVINF, it appears to be based on the information statistic and consequently is non combinatorial.

Re-allocation is possible by removing each element in turn and

calculating its dissimilarity with all groups including the one from which it was removed. Should it be found that a smaller dissimilarity occurs with another group, this element is deemed to be misclassified and is re-allocated to the new group. Dissimilarity measures are based on the Canberra metric (p. B3).

4. Diagnostic Programmes - GROUPER (Lance, Milne and Williams - 1968)

GROUPER is used to follow a classificatory programme. It calculates the contribution of each attribute towards the fusion of groups in the classification, and lists these attribute values in order of descending importance. In all cases, it was used to follow MULTBET but was not available for CLASS.

5. Ordination Programmes - GOWER (Gower - 1967)

The analysis carried out by GOWER enables the relationship between species, measured for a number of variates, to be visualized readily without confusion from a mass of figures.

The programme carries out a principal co-ordinate analysis using the dissimilarity matrix generated from the classificatory programme. The dissimilarity values are converted to distance measures and from these, a matrix of eigenvectors are calculated which represent the co-ordinates of the species on their principal axes. (For a mathematical treatment of this procedure, see Gower - 1967, Blackith and Reyment - 1971, and Sneath and Sokal - 1973). These co-ordinates can be plotted in a two dimensional field, and providing the variance encompassed by the first two or three eigenvectors is high, there is relatively little distortion from this treatment i.e. from the representation of points (species) from a multidimensional space into a two dimensional space. The amount of distortion can be checked by the superimposition of a minimum spanning tree (see below) over the co-ordinate plots.

6. Minimum Spanning Tree (Gower and Ross - 1969)

The minimum spanning tree (MST) is that set of straight line segments joining pairs of points such that

(i) no closed loops occur,

(ii) each point is visited by at least one line

and (iii) the tree is connected by the minimum total length.

7. Discussion of Procedures

All classificatory programmes used in these analyses share at least one shortcoming - they are unable to distinguish between the presence of common and rare attributes and between the absence of these. Such distinctions are important in taxonomy, but as yet, no programme is available which deals with them satisfactorily.

A second problem common to these programmes, with the exception of REMUL, involves the inability to deal with misclassified species. For example, in both CLASS and MULTBET, species are fused when the level of information is at its lowest and the possibility of misclassification is high. Consequently, a species may join a group very early in the fusion process but as more species are added, it becomes less like the group as a whole, and could probably be better accomodated elsewhere. However, there is no means of re-allocating the misclassified species to another group. A similar disadvantage is apparent in the divisive programme DIVINF. Although divisions are made on the basis of associated information (see p. B6), the choice of division at any level is restricted by the distribution patterns of the attributes. For example, in the illustration given below, divisions of the six species into groups I and II result in the greatest internal homogeneity within the two groups. However, species F would probably be better placed among group I species but since the division into groups is based on attribute 1, then the species must remain misclassified.

		attribute no.				
		1	2	3	4	
species	A	0	0	0	0	group I
	B	0	1	0	0	
	C	1	0	0	1	
	D	1	1	0	0	group II
	E	1	0	1	0	
	F	1	0	0	0	

This problem is overcome in the programme REMUL which permits re-allocation of misclassified species.

In all programmes using intensely clustering strategies, some degree of group-size dependence is observed. This problem has been discussed by Williams, Clifford and Lance (1970) for the two intensely clustering strategies used here i.e. the information statistic and the incremental sum of squares. They have shown that between groups of comparable size, the two techniques show a similar degree of group-size dependence *viz.* the measure between groups increases directly with

group size, providing the data favours neither strategy for any reason. Between individuals and groups, the effect of group-size also continues indefinitely with the information statistic but is asymptotic for the incremental sum of squares, having little effect at n = 5 and being negligible after n = 10. In consequence, the latter strategy is less likely to give rise to "non conformist" groups (see p. B12) often encountered in classifications using the information statistic.

The effect of group size is less apparent in weakly clustering strategies but these are not favoured generally in taxonomic work since they do not produce the discrete groups preferred by taxonomists. In addition, in the case of group average (= mean squared distance), Williams, Clifford and Lance (1970) have shown that this strategy is likely to be sensitive to aberrant data configuations e.g. skewed binary data where attributes may be lacked by most of the population. These workers have advocated the use of the information statistic when binary data is used but Burr (1970) has shown that the incremental sum of squares is sometimes preferrable.

The choice of similarity measure used in these programmes is also open to some debate. The main problem lies with deciding if common absences between species is representative of similarity in the same manner as joint presences. At present, it is not possible to determine this with biochemical data. Species may have the potential to accumulate certain compounds i.e. they possess the necessary enzymes, but are unable to do so because a metabolic block has occurred early in the biosynthetic pathway. In such cases, joint absences could be indicative of similarity. However, there is no means of distinguishing between this situation and where all the appropriate enzymes are entirely lacking. It may be expected however, that at lower taxonomic levels e.g. at the species level, joint absences are more likely to represent a degree of similarity than at the higher levels e.g. between families.

In data where many attributes are present in the group as a whole, but only occur sporadically among the species, the occurrence of joint absences will dominate the analysis, and if scored as a measure of similarity, they may completely distort the true relationship between the species. This difficulty is not restricted to biochemical data but is perhaps more obvious here than in other types of data.

In the procedures used here, the information statistic and the simple matching coefficient include joint absences as a measure of similarity whilst these are excluded by the Jaccard and Kulczynski

coefficients.

The Kulczynski coefficient was included in this work because in certain circumstances e.g. see below, it shows greater sensitivity than either S_{SM} or S_J. In the hypothetical example below, four species have been scored for ten attributes and the similarity between the first two species, and between the second two, has been calculated.

		attribute no. 1 2 3 4 5 6 7 8 9 10	S_{SM}	S_J	S_K
species	A	1 1 1 1 0 0 0 0 0 0	0.6	0.5	0.75
	B	1 1 1 1 1 1 1 1 0 0			
	C	1 1 1 1 1 1 0 0 0 0	0.6	0.5	0.66
	D	1 1 1 1 0 0 1 1 0 0			

S_K considers species C and D are less similar than species A and B, whereas S_{SM} and S_J do not distinguish between the two cases. It may be argued in support of S_K, that species C and D, in accumulating several different compounds, have indicated some positive degree of dissimilarity. If, for example, species C were able to accumulate more than 6 compounds, the probability that the additional compounds would be different from those in species D would be expected to be higher than in the alternative situation given for species A and B. In the latter case, species A has indicated an inability to accumulate as many compounds as species B but has shown no other positive sign of dissimilarity. In practice, it is no more possible to show that this is a valid argument, than it is to conclusively demonstrate the correctness or otherwise, of considering joint absences as representative of similarity. For this reason, it is necessary to compare as many similarity measures as practicable.

Finally, some mention should be made of the treatment of ordered multi-state data. In this work, MULTBET was the only programme able to accept data in this form. The information statistic (the clustering strategy involved) is only able to discriminate between the various attribute states providing all are present in the comparison under consideration. However, in the early fusions, attribute states will be missing i.e. when individuals or small groups are fused. The effect of this is illustrated in the example below (p. B11). Three species have been scored for a single five-state attribute. (When absent, an attribute is scored as 1, and when present, from 2 - 5 in order of increasing concentration.)

For a single character -

		character state 1	2	3	4	5
species	A	1	0	0	0	0
	B	0	0	0	0	1

$\Delta_I = 2ln2$

		character state 1	2	3	4	5
species	B	0	0	0	0	1
	C	0	0	0	1	0

$\Delta_I = 2ln2$

Species A and B are very dissimilar, with the attribute being absent in the first and very prominent in the second. On the other hand, the difference between species B and C is very slight. However, the information statistic treats both cases as similar and until groups are formed containing all character states, the procedure is continually subject to the same error. Even after all attribute states are present for all of the scored attributes, there is no means of re-allocating any misclassified species. In my opinion, the inaccuracy introduced by this treatment is sufficient to cast serious doubts on any classification resulting from the procedure.

GLOSSARY OF NUMERICAL TERMS

The definitions listed here have been taken from Lance and Williams (1967b), Williams (1971) and from introductory notes obtained from the C.S.I.R.O., Division of Computing, Hobart.

Agglomerative - An agglomerative classification is one that proceeds by successive fusion, beginning with the individuals and ending with the complete population.

Binary - Data in which the attributes occur in only two states i.e. present or absent. (= qualitative data)

Combinatorial - Once the inter-individual dissimilarity measures have been calculated, all future individual-group or group-group measures can be calculated from them, and the original data is no longer required.

Divisive - A divisive classification progressively splits the population dichotomously into smaller groups.

Group-size dependance - The dissimilarity measure is dependent on the number of individuals in the groups. As groups become larger, it becomes more difficult for individuals or smaller groups, to fuse with them.

Inverse - An inverse classification is one in which the characters are classified into groups.

Monothetic - Each division in the classification is based on a single attribute.

Non combinatorial - The original data is required throughout the computation (see combinatorial).

Non conformist groups - A group of outlying individuals having little in common with each other or with the rest of the population.

Normal - A normal classification is one in which individuals are classified into groups depending on the attributes they possess.

Ordered multi-state - The characters exist in more than 2 states such that the states are ranked e.g. absent, rare, common, abundant. With disordered multi-state data, the states are not ranked so that none is considered intermediate e.g. yellow, blue, red.

Polythetic - Each division or fusion in the classification is based on all attributes.

Qualitative - Data in two states (= binary).

Fig. B1. Dissimilarity matrices obtained for *Epacris* from MULTBET, CLASS (S_J) and CLASS (S_K).

MULTBET

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
E.acuminata	1	0																	
E.barbata	2	24	0																
E.corymbiflora	3	36	28	0															
E.exerta	4	20	12	32	0														
E.glabella	5	20	20	40	8	0													
E.gunnii	6	32	40	44	36	28	0												
E.heteronema	7	32	24	12	28	36	48	0											
E.impressa	8	32	40	52	36	28	8	48	0										
E.lanuginosa	9	40	24	20	28	36	48	16	48	0									
E.marginata	10	32	16	28	28	28	32	24	32	24	0								
E.mucronulata	11	36	20	40	32	40	52	28	52	36	36	0							
E.myrtifolia	12	32	16	28	28	36	48	24	48	24	16	28	0						
E.obtusifolia	13	20	44	56	32	24	12	52	12	52	36	56	52	0					
E.serpyllifolia	14	36	20	8	32	40	44	12	52	12	20	32	20	56	0				
E.stuartii	15	12	12	32	8	8	36	28	36	36	28	32	28	32	32	0			
E.tasmanica	16	24	0	28	12	20	40	24	40	24	16	20	16	44	20	12	0		
E.virgata	17	12	12	32	8	8	36	28	36	36	28	32	28	32	32	0	12	0	
*E.*var.*Davies Ck.*	18	48	40	36	44	52	64	24	64	40	48	36	32	68	36	44	40	44	0

CLASS (S_J)

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
E.acuminata	1	0																	
E.barbata	2	67	0																
E.corymbiflora	3	69	58	0															
E.exerta	4	63	43	67	0														
E.glabella	5	71	71	83	40	0													
E.gunnii	6	80	91	79	90	88	0												
E.heteronema	7	67	54	25	64	82	86	0											
E.impressa	8	80	91	87	90	88	29	86	0										
E.lanuginosa	9	71	50	36	58	75	80	31	80	0									
E.marginata	10	80	50	58	78	88	80	55	80	50	0								
E.mucronulata	11	69	45	63	67	83	87	50	87	56	69	0							
E.myrtifolia	12	67	40	50	64	82	86	46	86	43	40	50	0						
E.obtusifolia	13	63	100	93	89	86	43	93	43	87	90	93	93	0					
E.serpyllifolia	14	69	45	17	67	83	79	25	87	23	45	53	38	93	0				
E.stuartii	15	50	50	73	40	50	100	70	100	75	88	73	70	100	73	0			
E.tasmanica	16	67	0	59	43	71	91	55	91	50	50	45	40	100	45	50	0		
E.virgata	17	50	50	73	40	50	100	70	100	75	88	73	70	100	73	0	50	0	
*E.*var.*Davies Ck.*	18	75	67	53	73	87	89	40	89	56	75	53	50	94	53	79	67	79	0

CLASS (S_K)

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
E.acuminata	1	0																	
E.barbata	2	50	0																
E.corymbiflora	3	48	36	0															
E.exerta	4	45	27	42	0														
E.glabella	5	50	50	58	20	0													
E.gunnii	6	67	83	61	82	75	0												
E.heteronema	7	47	33	14	40	57	73	0											
E.impressa	8	67	83	74	82	75	17	73	0										
E.lanuginosa	9	50	25	22	29	38	63	18	63	0									
E.marginata	10	67	33	36	63	75	67	33	67	25	0								
E.mucronulata	11	48	23	45	42	58	74	33	74	39	48	0							
E.myrtifolia	12	47	20	33	40	57	73	30	73	27	20	33	0						
E.obtusifolia	13	45	100	85	80	73	27	85	27	72	82	85	85	0					
E.serpyllifolia	14	48	23	9	42	58	61	14	74	13	23	36	24	85	0				
E.stuartii	15	25	25	36	20	33	100	35	100	38	75	36	35	100	36	0			
E.tasmanica	16	50	0	36	27	50	83	33	83	25	33	23	20	100	23	25	0		
E.virgata	17	25	25	36	20	33	100	35	100	38	75	36	35	100	36	0	25	0	
*E.*var.*Davies Ck.*	18	52	40	35	46	60	76	23	76	38	52	35	31	86	35	39	40	39	0

MULTBET

		1	2	3	4	5	6	7	8	9	10	11
C.abietina	1	0										
C.dealbata	2	38	0									
C.divaricata	3	27	50	0								
C.glauca	4	27	46	36	0							
C.juniperina	5	32	52	27	38	0						
C.nitida	6	23	32	32	32	30	0					
C.parvifolia	7	29	55	23	38	25	34	0				
C.petiolaris	8	25	41	27	30	36	16	32	0			
C.straminea	9	30	57	39	14	41	36	41	34	0		
*C.*var.*intermedia*	10	32	30	45	41	43	20	46	25	45	0	
*C.*var.*pendulosa*	11	30	46	25	36	38	36	41	30	39	34	0

CLASS (S_J)

		1	2	3	4	5	6	7	8	9	10	11
C.abietina	1	0										
C.dealbata	2	78	0									
C.divaricata	3	56	85	0								
C.glauca	4	56	81	63	0							
C.juniperina	5	58	81	47	60	0						
C.nitida	6	54	69	62	62	55	0					
C.parvifolia	7	52	82	41	58	40	58	0				
C.petiolaris	8	54	77	52	57	59	38	53	0			
C.straminea	9	55	84	61	28	59	61	58	56	0		
*C.*var.*intermedia*	10	78	77	86	82	77	55	79	61	78	0	
*C.*var.*pendulosa*	11	71	93	56	71	68	77	70	65	69	86	0

Fig. B2. Dissimilarity matrices obtained for *Cyathodes* from MULTBET and CLASS (S_J).

APPENDIX C

Species	Source
Subfamily Epacrideae	
Archeria comberi Melville	Tas.
A.eriocarpa Hook. f.	Tas.
A.hirtella (Hook. f.) Hook. f.	Tas.
A.serpyllifolia Hook. f.	Tas.
A.traversii Hook. f.	N.Z.
Dracophyllum milliganii Hook. f.	Tas.
D.minimum F.Muell.	Tas.
D.recurvum Hook. f.	N.Z.
D.secundum R.Br.	N.S.W.
D.strictum Hook. f.	N.Z.
D.uniflorum Hook. f.	N.Z.
Epacris acuminata Benth.	Tas.
E.barbata Melville	Tas.
E.brevifolia Stapf	N.S.W.
E.corymbiflora Hook. f.	Tas.
E.crassifolia R.Br.	N.S.W.
E.exerta R.Br.	Tas.
E.glabella S.J.Jarman*	Tas.
E.gunnii Hook. f.	Tas., N.S.W.
E.heteronema Labill.	Tas.
E.impressa Labill.	Tas., Vic.
E.lanuginosa Labill.	Tas.
E.longiflora Cav.	N.S.W.
E.marginata Melville	Tas.
E.microphylla R.Br.	N.S.W.
E.mucronulata R.Br.	Tas.
E.myrtifolia Labill.	Tas.
E.obtusifolia Sm.	Tas., N.S.W.
E.paludosa R.Br.	N.S.W.
E.pauciflora A.Rich.	N.Z.
E.petrophila Hook. f.	Tas.
E.pulchella Cav.	N.S.W.
E.reclinata A.Cunn.	N.S.W.
E.rigida Sieb. ex Spreng.	N.S.W.
E.serpyllifolia R.Br.	Tas.
E.stuartii Stapf	Tas.
E.tasmanica W.M.Curtis	Tas.
E.virgata Hook. f.	Tas.
E. var. *Davies Ck.**	Tas.
E. var. *New South Wales**	Tas.
Lebetanthus americanus Endl.	South America
Prionotes cerinthoides (Labill.) R.Br.	Tas.
Richea acerosa (Lindl.) F.Muell.	Tas.
R.angustifolia B.L.Burtt	Tas.
R.continentis B.L.Burtt	N.S.W.
R.curtisii A.Gray	Tas.

Table C1. A list of species considered in this research.

(Tas. = Tasmania, N.S.W. = New South Wales, Vic. = Victoria, N.Z. = New Zealand.)

* Description unpublished but given in pp. C5 - C11.

Species	Source
Richea dracophylla R.Br.	Tas.
R.gunnii Hook. f.	Tas.
R.milliganii (Hook. f.) F.Muell.	Tas.
R.pandanifolia Hook. f.	Tas.
R.procera (F.Muell.) F.Muell.	Tas.
R.scoparia Hook. f.	Tas.
R.sprengelioides (R.Br.) F.Muell.	Tas.
Sprengelia distichophylla (Rodw.) Curtis	Tas.
S.incarnata Sm.	Tas., N.S.W.
Woollsia pungens F.Muell.	N.S.W.
Subfamily Styphelieae	
Acrotriche divaricata R.Br.	N.S.W.
A.serrulata (Labill.) R.Br.	Tas., N.S.W., Vic.
Astroloma humifusum (Cav.) R.Br.	Tas., Vic.
A.pinifolium (R.Br.) Benth.	Tas.
Brachyloma ciliatum (R.Br.) Benth.	Tas.
B.daphnoides (Sm.) Benth.	N.S.W,
B.depressum Benth.	Tas.
Cyathodes abietina R.Br.	Tas.
C.dealbata R.Br.	Tas.
C.divaricata Hook. f.	Tas.
C.empetrifolia Hook. f.	N.Z.
C.glauca Labill.	Tas.
C.juniperina (Forst.) Druce	Tas., N.Z., Vic.
C.nitida S.J.Jarman	Tas.
C.parvifolia R.Br.	Tas.
C.petiolaris (DC) Druce	Tas.
C.straminea R.Br.	Tas.
C. var. *intermedia**	Tas.
C. var. *pendulosa**	Tas.
Leucopogon amplexicaulis (Rudge) R.Br.	N.S.W.
L.australis R.Br.	Tas.
L.collinus (Labill.) R.Br.	Tas.
L.collinus var. *alpina**	Tas.
L.ericoides (Sm.) R.Br.	Tas., N.S.W., Vic.
L.ericoides var. *coastal**	Tas.
L.esquamatus R.Br.	N.S.W.
L.fasciculatus A.Rich.	N.Z.
L.hookeri Sond.	Tas.
L.juniperinus R.Br.	N.S.W.
L.lanceolatus (Sm.) R.Br.	N.S.W.
L.maccraei F.Muell.	N.S.W.
L.microphyllus Spreng.	N.S.W.
L.milliganii (F.Muell.) Rodw.	Tas.
L.parviflorus (Andr.) Lindl.	Tas., Vic.
L.setiger Spreng.	N.S.W.
L.stuartii F.Muell.	Tas., N.Z.
L.virgatus (labill.) R.Br.	Tas., Vic.
Lissanthe montana R.Br.	Tas.
L.sapida R.Br.	N.S.W.
L.strigosa (Sm.) R.Br.	N.S.W.

Table C1 cont'd.

Species	Source
Monotoca elliptica (Sm.) R.Br.	Tas.
M.empetrifolia R.Br.	Tas.
M.glauca (Labill.) Druce	Tas.
M.linifolia (Rodw.) W.M.Curtis	Tas.
M.scoparia (Sm.) R.Br.	Tas., N.S.W., Vic.
M.submutica (Benth.) S.J.Jarman*	Tas.
M. var. *L.Nicholls**	Tas.
M. var. *National Park**	Tas.
Pentachondra ericaefolia Hook. f.	Tas.
P.involucrata R.Br.	Tas.
P.pumila (Forst.) R.Br.	Tas., N.S.W.
Styphelia adscendens R.Br.	Tas.
S.laeta R.Br.	N.S.W.
S.triflora Andr.	N.S.W.
S.tubiflora Sm.	N.S.W.
Trochocarpa cunninghamii (DC) W.M.Curtis	Tas.
T.disticha (R.Br.) Spreng.	Tas.
T.gunnii (Hook. f.) Benth.	Tas.
T.laurina R.Br.	N.S.W.
T.thymifolia (R.Br.) Spreng.	Tas.

Table C1 cont'd.

SPECIES DESCRIPTIONS

The form of the descriptions given here follows that of Curtis (1963) and recommended by Stearn (1966).

In many cases, the plants described here are named by the location from which they were first discovered. This was done simply as a matter of convenience, and to my knowledge, these names have never been published and consequently have no official recognition. Exceptions include the three species whose taxonomic status is clear i.e. *Cyathodes nitida, Epacris glabella* and *Monotoca submutica*. Names chosen for these species are intended to be meaningful and will be retained for publication.

1. *Cyathodes nitida* S.J.Jarman.

Frutex humilis, 15 - 40 cm. altus, ramis patentibus et erectis, similis habitu *Cyathodi petiolari*. *Caules* juvenes minute pubescentes, vetustiores scabri. *Folia* alterna in caulibus novellis sub-erecta et conferta sed in vetustioribus saepe laxa et irregulariter patentia; lamina integra 4 - 10 mm. longa (acumine pungenti incluso) et 1.5 - 2 mm. lata angusta-oblonga usque angusta-lanceolata, margo valde recurvus, basis rotundata, apex acumine gracili pungenti 0.5 - 1 mm. longo sed in foliis primis cuiusque anni formatus acutatus et scariosus sed non pungens, supra plus minusve nitida subtus hebetata sed non glauca, parallelinerva subtus 3 nervis omnibus simplicibus, si supra apparet tantum in forma sulsi solitarii non profundi; petiolus minute puberulus 1 - 1.5 mm. longus. *Flores* solitarii in axillis foliorum versus extremitates ramorum. *Bractae* ciliatae, bracta suprema calyce circa 3-plo brevior. *Sepala* late elliptica 2 - 3 mm. longa apice rotundata. *Corolla* alba; tubus calycem vix superans bulbosus infra pilis brevibus, lobis patentibus tubo brevioribus plerumque glabris vel interdum pilis sparsis. *Antherae* semi-inclusae. *Discus* 5-lobus. *Ovarium* 5-loculare. *Fructus* drupaceus 2.5 - 4 mm. profundus 4 - 8 mm. in diametro depressissimus ruber-niger facie laevi nitida.

A low shrub resembling *C.petiolaris* in habit, with branches spreading and erect, 15 - 40 cm. high. Young stems puberulent, older ones with rough, scaly bark. Leaves alternate, sub-erect and crowded on young stems but often loose and spreading irregularly on older stems; blade entire, 4 - 10 mm. long (including the pungent point), 1.5 - 2 mm.

broad, narrow-oblong - narrow lanceolate, margin recurved, base rounded, apex forming a slender pungent point, 0.5 - 1 mm. long, but on the first formed leaves of each year's growth acute and scarious but not pungent, the upper surface somewhat shiny, the lower surface dull but not glaucous, venation parallel with 3 unbranched veins on the lower surface, if present on the upper surface then represented by a single, shallow groove; petiole minutely puberulent, 1 - 1.5 mm. long. Flowers solitary in the axils of leaves towards the ends of the branches. Bracts ciliate, the uppermost scarcely a third the length of the calyx. Sepals broadly elliptical, 2 - 3 mm. long, ciliate with rounded apex. Corolla white, the tube about as long as the calyx, bulbous, with short hairs inside, lobes shorter than the tube, spreading, narrowly imbricate at the base, usually glabrous but on some plants with a few scattered hairs. Anthers half exerted. Disk of 5 lobes. Ovary 5 locular. Fruit a drupe, very depressed, 2.5 - 4 mm. deep, 4 - 8 mm. in diameter, black-red, the surface smooth, shiny.

Holotype: Lake Augusta, Tasmania, R.K.Crowden and S.J.Jarman, 10.11.1970 (University of Tasmania Botany Department Herbarium, HO).

Cyathodes nitida is endemic to Tasmania and is known only from the Central Plateau, around L.Augusta (at an altitude greater than 1100 m.). In this region, it grows in shallow stony soils, in close association with *Monotoca empetrifolia*. These two form part of the low shrubbery in an open heathland dominated by the larger shrub, *Orites acicularis* (Proteaceae).

C.nitida flowers during October and November, with the fruits ripening in February-March, and remaining on the plant until the onset of winter (May-June).

The first known collection of this species was in 1970 by Dr.R.K.Crowden and myself. Drawings are shown in Fig. 22, p. 143.

2. *Cyathodes* var. *intermedia*

A low shrub, closely resembling *C.petiolaris* with branches spreading but ascending at the tips, 20 - 50 cm. high. Leaves spreading or half erect, narrow-elliptical, 1 - 1.5 cm. long, 2 - 3 mm. wide, apex with a hard pungent point or on the young leaves of each year's growth with a scarious tip, upper surface green, lower surface with 3 or occasionally 5 unbranched parallel veins, convex with the margin recurved, petiole

distinct, c. 1 mm. long. Flowers solitary or 2 or 3 together in the axils of leaves towards the ends of the branches. Sepals ovate, 2 - 3 mm. long, ciliate. Corolla white, tube slightly longer than the calyx, lobes shorter than the tube with the inner surface bearded at least in the lower half. Anthers included. Disk thick, annular. Ovary glabrous, 5-locular. Drupe red, flattened, c. 0.5 cm. in diameter, glabrous.

This variety was collected from Mt.Hamilton and Frenchman's Cap (on the West Coast) and has been recorded from the Denison Range (from unidentified herbarium specimens - Botany Department Herbarium).

C. var. *intermedia* flowers in spring (October and November).

3. *Cyathodes* var. *pendulosa*

A small erect shrub, 0.5 - 1 m. high, resembling *C.divaricata* in habit, with branches clustered along the stem, the branchlets villous. Leaves alternate and loose on primary stems but crowded and often with the petioles twisted, appearing distichous, on the short lateral branches, blade reflexed or spreading, entire, convex, the margin minutely barbed, 3 - 8 mm. long (including the petiole and the point at the apex), 0.5 - 1.5 mm. wide, apex tapering to a fine pungent point c. 0.5 mm. long, upper surface green, lower surface glaucous and striate with 3 - 5 unbranched parallel veins, base narrowed into a short petiole, c. 1 mm. long. Flowers solitary, terminal, rarely axillary, pendulous, pedicels 2 - 4 mm. long. Bracts imbricate, more closely overlapping in the male flowers than in the female flowers, ciliolate, the uppermost c. half the length of the calyx. Sepals elliptical, with rounded apex, c. 2 mm. long, ciliolate. Corolla white, glabrous, 3 - 4 mm. long, lobes shorter than the tube, spreading (male) or half spreading (female). Anthers half exerted. Disk of 5 scales or lobes. Ovary 5-locular. Fruit a drupe, 0.5 - 0.9 mm. deep, 0.7 - 1.2 cm. in diameter, pink or red.

The attachment of fruit tends to be terminal on the short, lateral branches. These are often recurved, or spreading but having flowers with recurved pedicels so that the fruit appears pendulous when it forms.

C. var. *pendulosa* was collected from the foothills of Ben Lomond (north eastern Tas.) where it was growing among rocks in an open Eucalypt forest (*Eucalyptus delagatensis*). The extent of its flowering season is not known but flowers were present in May and June when it was

collected. The fruit is persistent on the shrub so that both flowers and fruit are present at the same time.

The first known collection of this species was made by Mr.R.Shepherd (Botany Department).

4. *Epacris glabella* S.J.Jarman

A small shrub, 50 - 100 cm. high, with slender erect branches, the branchlets glabrous. Leaves with a short petiole, half spreading, thick, shiny, ovate, 5 - 7 mm. long (including the petiole), 2.5 - 3 mm. wide, apex obtuse, margin smooth, upper surface dark green, flat or slightly concave, lower surface lighter green, slightly keeled particularly near the apex, 1 - 3 indistinct ribs, petiole c. 1 mm. long. Flowers solitary, axillary and often extending along the branches. Bracts and sepals ovate, sepals c. 4 mm. long. Corolla white, tube funnel-shaped, shorter than, or equal to the calyx, lobes as long as the tube. Anthers exerted. Disk of 5 blunt lobes. Ovary 5-locular, style longer than the calyx. Fruit a capsule.

This species is endemic, and is known only from Dreadnought Hill (West Coast) where it grows in an open heath on serpentine soil. It flowers in September and October.

E.glabella has not been previously recorded, the first known collection being obtained by Dr.R.Crowden and myself in 1971.

5. *Epacris* var. *Davies Creek*

A slender shrub up to 2 m. high, with branchlets pubescent. Leaves narrow-obovate or narrow to broadly ovate, 3 - 5 mm. long and 2 - 3 mm. wide in the shorter leafed form, 5 - 8 mm. long and 2 - 3 mm. wide in the longer leafed form, apex acute with a short, sharp mucronate point, base rounded into a short petiole, margin minutely serrulate and very narrowly hyaline in the outer half, upper surface flat or slightly concave, the lower surface with a shallow keel in the outer half, venation obscure or with 1 - 3 indistinct veins on the undersurface. Flowers solitary, axillary towards the ends of the branches. Bracts and sepals ovate, acute, ciliolate; sepals 4 - 5 mm. long. Corolla white, the tube as long as or shorter than the calyx, lobes equal to the tube, broad, blunt. Anthers almost sessile, at the throat of the tube. Disk of 5 scales. Ovary 5-locular, style short, with the lower half bulbous. Fruit a capsule.

E. var. *Davies Ck.* appears to be fairly widespread on the West Coast and I have collected it from Mt.Arrowsmith pass (before road reconstruction), from the track to Frenchman's Cap and on the road between Granville Harbour and Zeehan. It has also been found on the Tyndall Range by members of the Geography Department (University of Tasmania).

E. var. *Davies Ck.* flowers in November and December.

6. *Epacris* var. *New South Wales*

A low shrub, 10 - 30 cm. high, with branchlets pubescent. Leaves half spreading, narrow elliptical, 5 - 9 mm. long, 1 - 1.5 mm. wide, with a short petiole c. 1 mm. long.

This species was seen only at Lawson in New South Wales. It was found among rocks on the shady side of a sheltered gully. Flowers of this species were not examined

E. var. *N.S.W.* was first obtained from Mrs. E. Reilly in New South Wales.

7. *Leucopogon ericoides* var. *coastal*

This species closely resembles *L.ericoides* but differs in several leaf characters including a blunt leaf apex, a shorter length-breadth ratio, more revolute margins and more crowded leaves. In addition, the flowers are often solitary, and from some locations on the West Coast, are much larger than those of typical *L.ericoides*. The fruit is white and is more succulent than that of *L.ericoides*.

L.ericoides var. *coastal* is widespread in Tasmania and although it is particularly common in open heaths near the coast, it has also been collected from inland areas (e.g. L.Pedder beach).

8. *Monotoca submutica* (Benth.) S.J.Jarman

A tall shrub, 2 - 3 m. high, or sometimes a small tree up to 6 m. high, branchlets puberulent. Leaves sub-erect, elliptical to oblong or obovate, 6 - 12 mm. long (including the petiole), 2 - 3.5 mm. wide, flat or slightly convex with the margin scarcely recurved, upper surface green, lower surface glaucous, apex with a short callous tip, petiole distinct, c. 1 - 1.5 mm. long. Flowers axillary, frequently solitary but also in short spikes of 2 - 4 flowers, peduncle usually recurved with a variable number of bracts (2 - 7). Sepals broadly ovate, c.

1 mm. long, ciliate. Corolla white but quickly turning yellowish-white when removed from the plant, tube c. 1 mm. long, equal in length to the calyx, lobes longer than the tube. Anthers half exerted. Ovary unilocular. Disk of 5 lobes. Drupe spherical or oval, orange-red or less frequently yellow, up to 4 mm. deep.

M.submutica is widespread in southern and western Tasmania, growing in subalpine forest, where it is usually a low to medium shrub, or in wet sclerophyll associated with rainforests where it forms a small tree.

A more robust form of *M.submutica* is known from one location on the east coast.

9. *Monotoca* var. *Lake Nicholls*

A low loose shrub, 15 - 50 cm. high. Leaves alternate, loose, spreading, lamina broadly ovate, 3 - 6 mm. long, 2 - 4 mm. wide, upper surface green, lower surface glaucous, margin thickened and slightly recurved, apex rounded with a short, callous tip, petiole distinct, 1 - 2 mm. long, minutely puberulent. Flowers axillary, solitary or sometimes 2 or 3 together in the leaf axils towards the base of the short terminal branchlets. Calyx overlapped by 2 bracts. Sepals ovate, c. 1 mm. long, ciliolate. Corolla white, lobes longer than than the tube, spreading on male flowers but erect or sub-erect on the smaller female flowers. Anthers exerted on the male flowers but undeveloped on female flowers. Disk of 5 lobes. Ovary unilocular. Drupe ovoid, 2 - 3 mm. long, orange or yellow.

At present, this species is known only from subalpine forest on Mt.Field where it forms part of the low shrubbery beneath *Eucalyptus coccifera* and *E.johnstonii*.

10. *Monotoca* var. *National Park*

A low shrub, less than 1 m. high, with branchlets having short scattered hairs. Leaves sub-erect, or spreading, narrow-oblong to elliptical, 6 - 10 mm. long, 2 - 2.5 mm. wide, margin recurved, upper surface green, lower surface glaucous, apex with a short pungent point (but with a callous point in some West Coast forms), petiole distinct, often red, c. 1 mm. long. Flowers axillary, usually on short recurved peduncles with 2 - 4 flowers, but sometimes solitary. One bract and 2 bracteoles at the base of each flower, sepals ovate, ciliolate, c.

1 mm. long. Corolla white but sometimes tinged with pink, the tube c. 1 mm. long and equal to the calyx, the lobes equal to the tube. Filaments recurved, longer than the anthers. Ovary usually bilocular but sometimes 1 - 3 locular. Disk of 5 broadly ovate scales. Drupe white, pink or purple, spherical, 2 - 4 mm. in diameter.

M. var. *Nat.Pk.* was first collected from Mt.Field National Park where it was found growing with *Richea pandanifolia* and *Trochocarpa cunninghamii* in an open heath surrounded by rainforest. I have subsequently found it in sub-alpine forest near Mt.Rufus (western edge of the Central Plateau) and in open patches of rainforest on the slopes of Mt.Arrowsmith. Blunt leafed forms of the species have been recorded from Waratah and Lake St.Clair (on unnamed herbarium sheets - Botany Department Herbarium).

M. var. *Nat.Pk.* flowers in October - November, with the fruits ripening in February - April but falling quickly after maturation.

AN IDENTIFICATION KEY FOR THE EPACRIDACEAE IN TASMANIA

Species from the Epacridaceae have gained a reputation for being amongst the most difficult plants to identify in Tasmania. This is partly due to taxonomic problems within the group, and partly due to the difficulties encountered when keying out the species. The only keys available for the Tasmanian flora (Bentham - 1869, Rodway - 1903, Curtis - 1963) are phylogenetic keys which frequently necessitate the use of microscopic characters in order to key out families and genera as discrete units. While such keys are valuable in research, their shortcomings in field identifications are obvious, particularly when used by non taxonomists.

The problems involved in constructing simple "all-purpose" keys are well appreciated and this has not been attempted here. The alternative approach is to construct several keys, each for a particular purpose. With this in mind, I have designed a key specifically to assist with rapid, easy identifications in the field. The key is completely artificial and makes no attempt to show relationships among genera and species. The aim has been to find the shortest and easiest route to a particular species, irrespective of adjacent species which may belong to quite unrelated genera. Microscopic characters have been avoided wherever possible. Where species show considerable intra-specific variation, the variants are keyed out independently in order to avoid the use of minute characters.

Complete botanical descriptions for the majority of species included here are given by Curtis (1963). The description of *Richea curtisii* may be found in Gray (1971). Descriptions or information for other species and varieties have been included on pp. C5 - C11.

Botanical terminology used in the key is consistent with that of Curtis (1956, 1963) and Stearn (1966).

1. Corolla forming an operculum ..... 2.
   Corolla forming a tube with free lobes ..... 11.

2. Flowers solitary but forming short terminal heads ..... 3.
   Inflorescence compound (spike, raceme, panicle) ..... 6.

3. Flower head pendulous ..... *Richea milliganii*
   Flower head erect ..... 4.

4. Filaments equal to, or shorter than the calyx .. *R.acerosa*
   Filaments distinctly longer than the calyx (± 2×) ..... 5.

5. Filaments uniformly thick, operculum acuminate.. *R.sprengelioides*
   Filaments thickened below the anthers, operculum tapering gradually from the widest point ..... *R.procera*

6. Inflorescence axillary ..... *R.pandanifolia*
   Inflorescence terminal ..... 7.

7. Sheathing leaf base narrowing abruptly, forming a distinct shoulder ..... 8.
   Lamina tapering gradually from the sheathing base ..... 9.

8. Leaves on older branches falling cleanly from the stem ..... *R.scoparia*
   Leaves tearing unevenly, with parts of the leaf base remaining after leaf abscission ..... *R.angustifolia*

9. Leaves < 6 cm. long ..... *R.gunnii*
   Leaves > 6 cm. long ..... 10.

10. Filaments of the stamens barely reaching the stigma ..... *R.curtisii*
    Filaments c. twice the length of the style ..... *R.dracophylla*

11. Leaf base sheathing the stem ..... 12.
    Leaves sessile, petiolate or stem clasping at the base but not sheathing ..... 15.

12. Sepals more than twice the length of the corolla tube ..... 13.
    Sepals less than, or equal to the tube..... 14.

13. Leaves spiral ..... *Sprengelia incarnata*
    Leaves distichous ..... *S.distichophylla*

14. Flowers solitary ..... *Dracophyllum minimum*
    Inflorescence compound (panicle) ..... *D.milliganii*

15. Flowers pink or red ..... 16.
    Flowers white, cream, yellow, green or orange (the outer surface of the lobes may be pink but the remainder of the corolla is white) ..... 27.

16. Leaf undersurface glaucous ..... *Lissanthe strigosa*
    Leaf undersurface green ..... 17.

17. Filaments free (anthers sometimes attached to the tube) ..... *Prionotes cerinthoides*
    Filaments fused to the corolla tube ..... 18.

18. Bracts overlapping the sepals ..... 19.
    Bracts not reaching the sepals ..... 23.

19. Flowers solitary ..... 20.
    Flowers forming a short spike ..... 21.

20. Corolla glabrous, 5 depressions at the base .... *Epacris impressa*
    Corolla hairy, depressions absent from the base. *Astroloma humifusum*

21. Leaf undersurface with a single midrib ..... *Trochocarpa thymifolia*
    Leaf undersurface with 3 - 7 unbranched veins ..... 22.

22. Young stems pubescent ..... *T.cunninghamii*
    Young stems glabrous ..... *T.disticha*

23. Ovary pubescent ..... *Archeria eriocarpa*
    Ovary glabrous ..... 24.

24. Leaf lamina > 0.4 cm. long, medium to tall rainforest shrub
    ..... *A.hirtella*
    Leaf lamina < 0.4 cm. long, medium to low alpine shrub
    ..... *A.comberi*

25. Corolla yellow or orange and > 0.6 cm. long ..... 26.
    Corolla white, cream or green, if yellow then < 0.6 cm. long. 27.

26. Anthers and filaments exerted (corolla yellow).. *Styphelia adscendens*
    Anthers and filaments enclosed (corolla orange). *Astroloma pinifolium*

27. Hairs present on the corolla lobes ..... 28.
    Corolla lobes glabrous (the tube may be hairy) ..... 57.

28. Corolla green ..... *Acrotriche serrulata*
    Corolla cream, white or white with pink lobes (outer surface) 29.

29. Flowers solitary ..... 30.
    Inflorescence of 2 or more flowers ..... 45.

30. Leaf undersurface glaucous ..... 31.
    Leaf undersurface green ..... 39.

31. Corolla lobes with long scattered hairs ..... 32.
    Hairs short and densely crowded on the corolla lobes ..... 34.

32. 3 - 5 parallel veins on the leaf undersurface .. *Cyathodes divaricata*
    > 5 parallel veins on the leaf undersurface ..... 33.

33. Leaf lamina > 1.2 cm. long, corolla lobes revolute. *C.glauca*
    Leaf lamina < 1.2 cm. long, corolla lobes spreading or slightly
    recurved ..... *C.straminea*

34. Veins branched (and not prominent) ..... 35.
    Veins unbranched and prominent ..... 36.

35. Style exerted beyond the calyx (after removal of the corolla),
    alpine shrub ..... *Leucopogon stuartii*
    Style shorter than the calyx, coastal shrub .... *Brachyloma ciliatum*

36. 1 - 3 veins on the leaf undersurface ..... 37.
    > 3 veins on the undersurface ..... 38.

37. Leaves flat with a single midrib ..... *Cyathodes dealbata*
    Margins recurved, 3 veins on the undersurface .. *C.* var. *intermedia*

38. Length-breadth ratio of leaves > 4:1, extreme coastal shrub
    ..... *C.abietina*
    Length-breadth ratio of leaves < 4:1, alpine shrub.. *C.petiolaris*

39. Leaf apex with a slender pungent point ..... 40.
    Leaf apex acute, obtuse and blunt ..... 42.

40. Anthers exerted ..... *Leucopogon esquamatus*
    Anthers enclosed or at the throat of the tube ..... 41.

41. Style exerted beyond the calyx (after removal of the corolla),
    alpine shrub ..... *Leucopogon stuartii*
    Style shorter than the calyx ..... *Brachyloma ciliatum*

42. Leaf margin ciliate or hairy ..... 43.
    Leaf margin smooth ..... 44.

43. Outer surface of the corolla tube covered with short dense hairs
    ..... *Pentachondra ericaefolia*
    Outer surface of the tube glabrous ..... *P.involucrata*

44. Prostrate alpine shrub, leaves < 0.7 cm. long, concave
    ..... *P.pumila*
    Erect lowland shrub, leaves > 0.7 cm. long, margins revolute
    ..... *Leucopogon ericoides*
    var. *coastal*

45. Leaves > 1.5 cm. long, at least 3 veins apparent on the upper surface
    for more than ½ the length of the lamina ..... 46.
    Leaves usually < 1.5 cm. long, venation not apparent on the upper
    surface or apparent only as a single groove ..... 48.

46. Leaves usually between 1.5 and 2.5 cm. long, the outer 2 veins on
    the upper surface disappearing just beyond the middle
    ..... *Leucopogon parviflorus*
    Leaves usually > 2.5 cm., 3 veins apparent along the whole length
    of the upper surface ..... 47.

47. Margins revolute, leaves with a papery texture, branchlets glabrous
    ..... *L.australis*
    Margins flat, leaf texture not as above, branchlets minutely
    pubescent (a hand lens is required) ..... *L.lanceolatus*

48. Leaf undersurface glaucous with the venation prominent ..... 49.
    Leaf undersurface green, veins not prominent ..... 50.

49. Veins branched ..... *L.hookeri*
    Veins unbranched ..... *Cyathodes petiolaris*

50. Inflorescence axillary along the stem ..... 51.
    Inflorescence terminal or axillary at the ends of the branches
    ..... 53.

51. Apex blunt ..... *Leucopogon ericoides* var. *coastal*
    Apex with a slender pungent point ..... 52.

52. Filaments and anthers exerted, leaves flat ..... *L.esquamatus*
    Filaments enclosed, anthers at the throat of the tube ..... *L.ericoides*

53. Corolla tube > 0.3 cm. long ..... *Archeria serpyllifolia*
    Corolla tube < 0.3 cm. long ..... 54.

54. Inflorescence pendulous ..... *Trochocarpa thymifolia*
    Inflorescence erect ..... 55.

55. Leaves involute ..... *Leucopogon virgatus*
    Leaves flat or revolute ..... 56.

56. Erect shrub in lowland heaths ..... *L.collinus*
    Prostrate shrub with slender wiry branches, alpine. *L.collinus* var. *alpina*

57. Leaf undersurface glaucous ..... 58.
    Leaf undersurface green ..... 68.

58. Flowers with 4 petals and sepals ..... 59.
    Flowers with 5 petals and sepals ..... 60.

59. Veins branched on the leaf undersurface..... *Monotoca linifolia*
    Veins unbranched ..... *M.empetrifolia*

60. Leaf apex blunt ..... 61.
    Leaf apex with a slender pungent point ..... 64.

61. Flowers in axillary spikes at the ends of the branches ..... *Lissanthe montana*
    Flowers in axillary spikes towards the base of the branchlets ..... 62.

62. Length-breadth ratio of the leaf < 2:1 ..... *Monotoca* var. *L.Nicholls*
    Length-breadth ratio of the leaf > 2:1 ..... 63.

63. Leaf margin recurved, width < 0.25 cm..... *M.*var.*National Park*
    Leaf margin flat, width > 0.25 cm. ..... *M.submutica*

64. Hairs present at the throat of the corolla tube, flowers solitary ..... *Brachyloma depressum*
    Corolla glabrous, flowers rarely solitary ..... 65.

65. Corolla tube longer than the lobes ..... *Monotoca scoparia*
    Corolla tube shorter than the lobes ..... 66.

66. Inflorescences axillary at the ends of the branches ..... *M.elliptica*
    Inflorescences axillary towards the base of the branchlets... 67.

67. Leaves < 0.25 cm. wide ..... *M.*var.*National Park*
    Leaves > 0.25 cm. wide ..... *M.glauca*

68. Flowers in short spikes ..... *Lissanthe strigosa*
Flowers solitary ..... 69.

69. Leaves > 0.7 cm. long ..... *Cyathodes juniperina*
Leaves < 0.7 cm. long ..... 70.

70. Petioles twisted on the young branches and the leaves appearing distichous, branches in annular clusters ..... *C.*var.*pendulosa*
Petioles not twisted, leaves alternate and the branches not clustered ..... *C.parvifolia*

70. Hairs present at the throat of the corolla tube ..... 71.
Tube glabrous or with very short inconspicuous hairs which are not visible without opening the tube ..... 73.

71. Inflorescence erect ..... *Archeria serpyllifolia*
Inflorescence pendulous ..... 72.

72. Young branchlets glabrous ..... *Trochocarpa disticha*
Young branches pubescent ..... *T.cunninghamii*

73. Bracts distant from the sepals ..... 74.
Bracts overlapping the sepals ..... 75.

74. Ovary pubescent ..... *Archeria eriocarpa*
Ovary glabrous ..... *A.hirtella*

75. Style tapering from the ovary, corolla lobes not overlapping or indistinctly so..... 76.
Style inserted in a central depression of the ovary, corolla lobes clearly overlapping at the base ..... 77.

76. Leaf apex with a slender pungent point ..... *Cyathodes nitida*
Leaf apex blunt ..... *Trochocarpa gunnii*

77. Style equal to, or longer than the calyx (remove the corolla) 78.
Style shorter than the calyx ..... 89.

78. Anthers sessile or sub-sessile but not exerted beyond the tube ..... 79.
Anthers and filaments exerted beyond the tube ..... 83.

79. Lobes shorter than ½ the length of the tube ..... 80.
Lobes longer than ½ the length of the tube (usually ± the length of the tube ..... 81.

80. 5 depressions or pits at the base of the corolla..*Epacris impressa*
Depressions absent from the base of the corolla...*E.obtusifolia*

81. Style with short hairs at the middle ..... *E.lanuginosa*
Style glabrous ..... 82.

82. Alpine or subalpine shrub, leaves subsessile and the venation indistinct ..... *E.paludosa*
Eucalypt or rainforest species, leaves distinctly petiolate, at least 3 veins apparent on the undersurface. *E.mucronulata*

83. Sepals villous ..... *E.barbata*
Sepals glabrous ..... 84.

84. Leaves thin, very concave (and stem clasping at the base) ..... *E.acuminata*
Leaves thick, flat or convex, not stem clasping ..... 85.

85. Leaves prickly, apex with a short fine point ..... 86.
Leaves blunt, often with a point at the apex but incurved and not prickly ..... 87.

86. Leaves mostly flat, very shiny, from exposed coastal heaths (Southport Bluff) ..... *E.stuartii*
Leaves mostly recurved, usually dull on the upper surface, present in sheltered coastal heaths ..... *E.tasmanica*

87. Length-breadth ratio of the lamina > 2:1 ..... *E.exerta*
Length-breadth ratio of the lamina < 2:1 ..... 88.

88. Young stems glabrous ..... *E.glabella*
Young stems pubescent ..... *E.virgata*

89. Corolla tube cylindrical ..... 90.
Corolla tube funnel shaped ..... 94.

90. Bracts and sepals hard, yellow-brown ..... *E.corymbiflora*
Bracts and sepals white, often tinged with red ..... 91.

91. Leaf margin hyaline, > 5 veins on the undersurface. *E.heteronema*
Leaf margin not hyaline, or indistinctly so, < 5 veins apparent on the undersurface ..... 92.

92. Length-breadth ratio of the lamina > 4:1 ..... *E.mucronulata*
Length breadth ratio of the lamina < 4:1 ..... 93.

93. Alpine or sub-alpine shrub ..... *E.serpyllifolia*
Rainforest shrub ..... *E.*var.*Davies Ck.*

94. Leaf base cordate, apex prickly ..... 95.
Leaf apex not cordate, apex blunt ..... 96.

95. Cordate base hyaline, leaves > 0.7 cm. long .... *E.marginata*
Cordate base green, leaves < 0.7 cm. long ..... *E.gunnii*

96. Alpine shrub, leaves < 0.3 cm. long ..... *E.petrophila*
Coastal shrub, leaves > 0.3 cm. long ..... *E.myrtifolia*

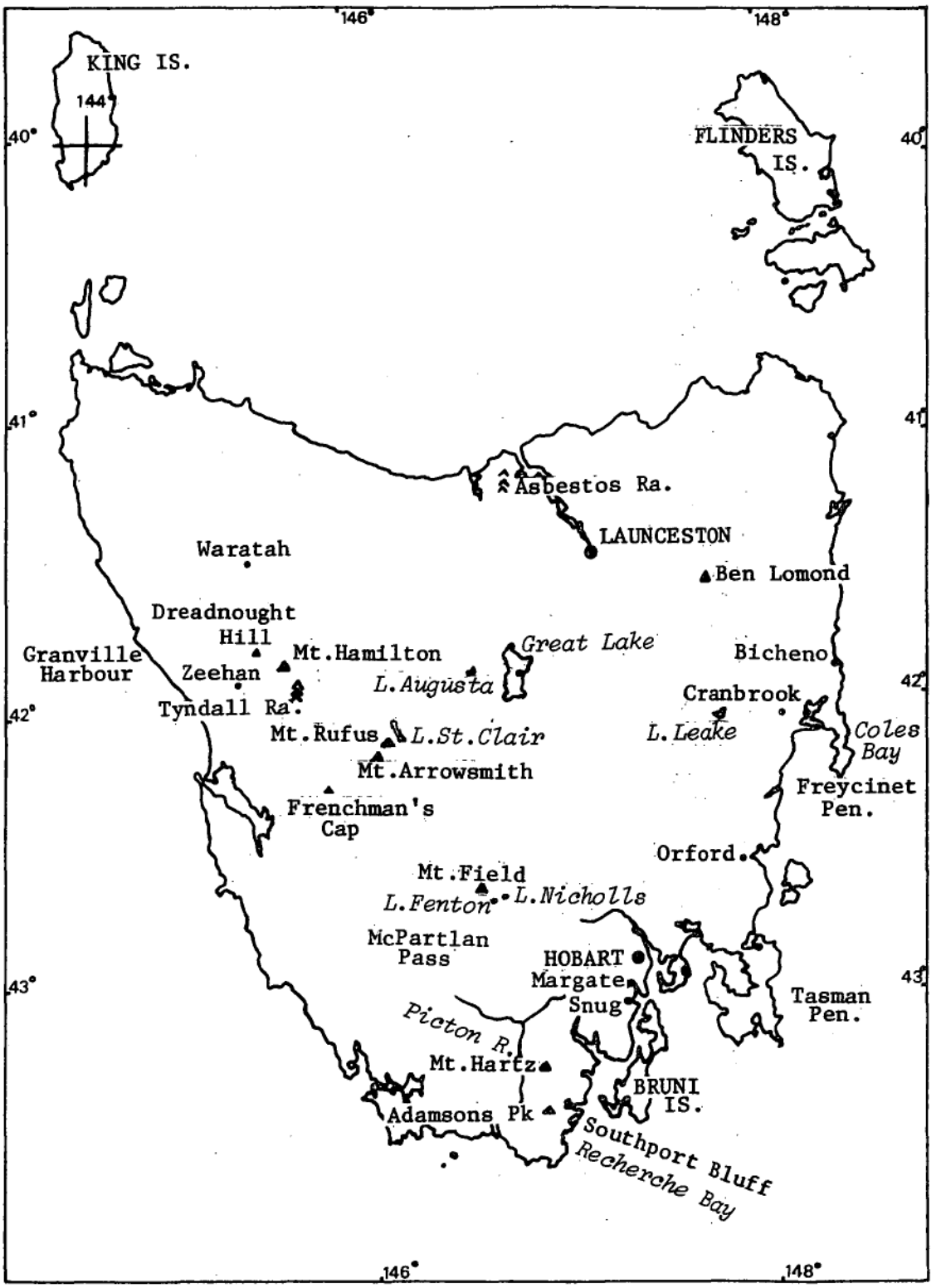


Fig. C1. A map of Tasmania.